http://dx.doi.org/10.1590/1678-4162-12962

Arq. Bras. Med. Vet. Zootec., v.75, n.6, p.1165-1170, 2023

Deoxinivalenol deactivation in wheat grains treated with ammonia gas and its effect on *Rattus norvegicus* diet

[*Desativação do desoxinivalenol em grãos de trigo tratados com gás amônia e seu efeito na dieta de* Rattus norvegicus]

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ABSTRACT

Fusarium head blight, also known as fusariosis, is caused by a fungus called *Fusarium graminearum* that produces the mycotoxin deoxynivalenol (DON). This toxin causes problems to human and animal health. Ammonia gas has been shown to be effective in deactivating mycotoxins. The objectives of this study were to evaluate the effectiveness of ammonia gas in the deactivation of DON in wheat grains, the effect of this treatment on its protein composition and the toxicity in rats fed with ammoniated wheat grains. Wheat samples were exposed to ammonia gas at concentrations of 0% (Control), 0.5%, 1% and 1.5%. It was observed that ammonia gas was effective in deactivating DON at concentrations of 1% and 1.5%. There was no difference in crude protein observed in the bromatological analysis of treated wheat grains in relation to the control. Regarding the toxicity of wheat grains treated with ammonia in the feeding of Wistar rats (*Rattus norvegicus*), no histopathological alterations were observed in the liver, kidneys, intestine and in the hematological profile. The results of this study demonstrated that the treatment of wheat with ammonia gas can contribute to the deactivation of DON without compromising its protein composition and animal health.

Keywords: desoxinivalenol, ammonia gas, animal health, nutrition

RESUMO

*A giberela, também conhecida como fusariose, é causada por um fungo chamado Fusarium graminearum, que produz a micotoxina desoxinivalenol (DON). Essa toxina gera problemas à saúde humana e animal. O gás amônia demonstrou ser eficaz na desativação das micotoxinas. Os objetivos deste estudo foram avaliar a eficácia do gás amônia na desativação do DON em grãos de trigo contaminados naturalmente, o efeito desse tratamento na sua composição proteica e a toxicidade em ratos alimentados com os grãos de trigo amonizados. Amostras de trigo foram expostas ao gás amônia nas concentrações de 0% (controle), 0,5%, 1% e 1,5%. Observou-se que o gás amônia foi eficaz na desativação do DON na concentração de 1% e 1,5%. Não houve diferença na proteína bruta observada na análise bromatológica dos grãos de trigo tratados em relação ao controle. Quanto à toxicidade dos grãos de trigo tratados com amônia fornecidos aos ratos Wistar (*Rattus norvegicus*), não foram observadas alterações histopatológicas do fígado, dos rins e do intestino e no perfil hematológico. Os resultados deste estudo demonstraram que o tratamento de trigo com gás amônia pode contribuir na desativação do DON sem comprometer sua composição proteíca e a saúde dos animais*.

Palavra-chave: desoxinivalenol, gás amônia, nutrição, saúde animal

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Submitted: December 31, 2022. Accepted: July 13, 2023.

INTRODUCTION

In Brazil, wheat is produced in regions with a subtropical and tropical climate that favor the development of the fungus *Fusarium graminearum*, responsible for the production of the toxin deoxynivalenol (DON) (Mesterházy, 2002).

About 94% of Brazilian wheat and 88% of imported wheat are positive in tests for the presence of DON (Pereira, *et al*, 2017; Calori-Domingues *et al*., 2007). Several countries have significant losses due to the presence of the DON toxin, such as the United States with a loss of 655 million dollars per year. Central Europe and North Asia also show losses with DON (Munkvold *et al*., 2019). DON can induce vomiting, diarrhea, immunosuppression, reduced protein synthesis and hemorrhage in animals and humans (Garda and Badiale-Fulong, 2008; Peraica *et al*. 1999).

Several scientific studies have already demonstrated that ammonization is the most effective process to inactivate mycotoxins in cereals and animal feed (Park et al, 1998). Treatment of maize kernels with ammonia was efficient in inactivating aflatoxins without reducing their nutritional value (Brekke *et al.*, 1977). Ammonia has nitrogen as the central atom that contains the most bonds. Meanwhile, DON has five electrons in the valence shell, plus one pair of electrons remaining on the central atom (Zeller *et al.*, 1991). When ammonia interacts with DON through Van der Waals forces, the remaining electron pair of the central DON atom breaks its benzene ring, forming two different and non-toxic compounds (Borràs-Valllverdú *et al*., 2020). In quantum mechanics, the action of Van der Waals forces can be understood by the fact that there is a finite probability that the electrons belonging to a given atom are found at infinity (Parsegian, 2005).

Thus, the objectives of this study were to evaluate the effectiveness of ammonia gas in deactivating DON in wheat grains, the effect of this treatment on the bromatological composition of the wheat grain and its toxicity in rats fed with the treated grains.

MATERIALS AND METHODS

This research was carried out in the College of Agronomy and Veterinary Medicine at the University of Passo Fundo in partnership with the companies Aveclean and Biotrigo Genetica.

The work was performed in three experiments: firstly, the deoxynivalenol deactivation by ammonia was verified through the enzyme-linked immunosorbent assay (ELISA); followed by crude protein quantification of the wheat samples, and lastly the *in vivo* test was carried out with rats to verify the toxicity of the treated wheat grains.

Experiment 1: wheat grains of the cultivar TBIO Audaz obtained from a commercial field during the 2019 cropping season in the municipality of Coxilha-RS were fractionated into 10kg samples. Samples were placed in 200-micron waterproof plastic bags and ammonia gas was injected at concentrations of 0.0%, 0.5%, 1% and 1.5% in 8 replicates. After seven days of ammonia gas application, the samples of each treatment were analyzed by the ELISA test, using the quantitative Veratox® kit to compare the DON reduction in relation to the control treatment.

Experiment 2: bromatological analysis of crude protein in wheat grains. Eight samples were collected from each treatment (0.0%, 0.5%, 1% and 1.5% of ammonia gas) and submitted to bromatological analysis with the Kjeldahl method to determine the amount of nitrogen. We used the equation CP= N x 6.25 to transform the nitrogen estimates into crude protein. Posteriorly, the composition of the samples treated with different concentrations of ammonia gas was compared in relation to the control treatment.

Experiment 3: *in vivo* test of Wistar rats fed with treated wheat grains. The project was submitted and approved by the ethics committee for the use of animals at the University of Passo Fundo (CEUA) under protocol 011/2020.

The *in vivo* test was performed with 32 thirty-day-old male Wistar rats. Rats were fed for 30 days with feed made from wheat treated with ammonia. The wheat was milled and then prepared in blocks to facilitate consumption. According to the density per cage proposed by the ethics committee, the animals were divided into five groups:

G1: control group with four rats fed with blocks of 100 grams of basal diet offered daily.

G2: four rats receiving 50 grams of feed blocks with control wheat without ammonia and 50 grams of basal diet.

G3: eight rats fed 100 grams of control blocks and 100 grams of blocks made with feed treated with 0.5% ammonia.

G4: eight rats fed 100 grams of control blocks and 100 grams of blocks made with feed treated with 1% ammonia.

G5: eight rats fed 100 grams of control blocks and 100 grams of blocks made with feed treated with 1.5% ammonia.

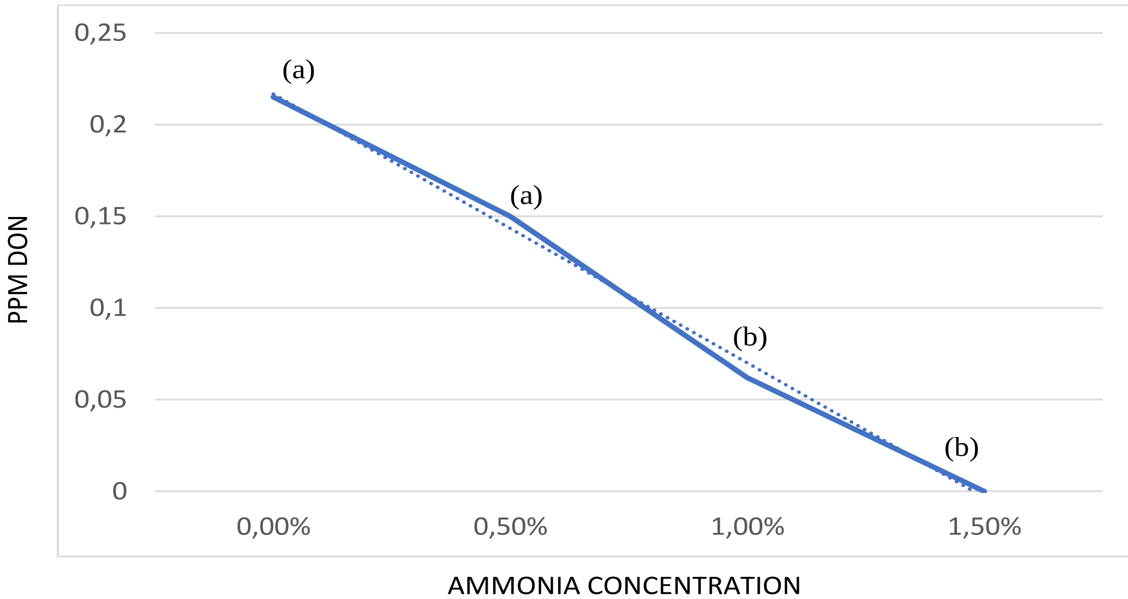
The blocks were prepared daily for the 30 days interval to ensure good feed quality. The proposed amount of cereal was weighed, mixed with water until consistency and offered on the top of the cage. The offer above the cage and not gavage was chosen in order to analyze the wheat organoleptic acceptance by the animals. The animals were also weighed every 3 days to verify weight gain and zootechnical performance. After this period, all animals were numbed with isoflurane, euthanized, and necropsied for further histopathological examination.

Small intestines, kidney, and liver were collected in the necropsy. The organs were placed in individual containers with 10% formalin and sent for histopathological examination. In addition, blood was collected and sent to the clinical analysis laboratory to perform tests to verify potential changes in liver enzymes following components were analyzed: alanine aminotransferase (ALT), urea, creatinine, alkaline phosphatase, cholesterol, triglycerides, total protein, albumin, and globulins.

The experiment was carried out in a completely randomized design (Oneill Mathews). Data were subjected to analysis of variance and treatment mean comparisons by Tukey's test.

RESULTS AND DISCUSSION

The results found in experiment 01 (Fig. 1) demonstrate the decrease in deoxynivalenol levels in wheat samples treated with different concentrations of ammonia.

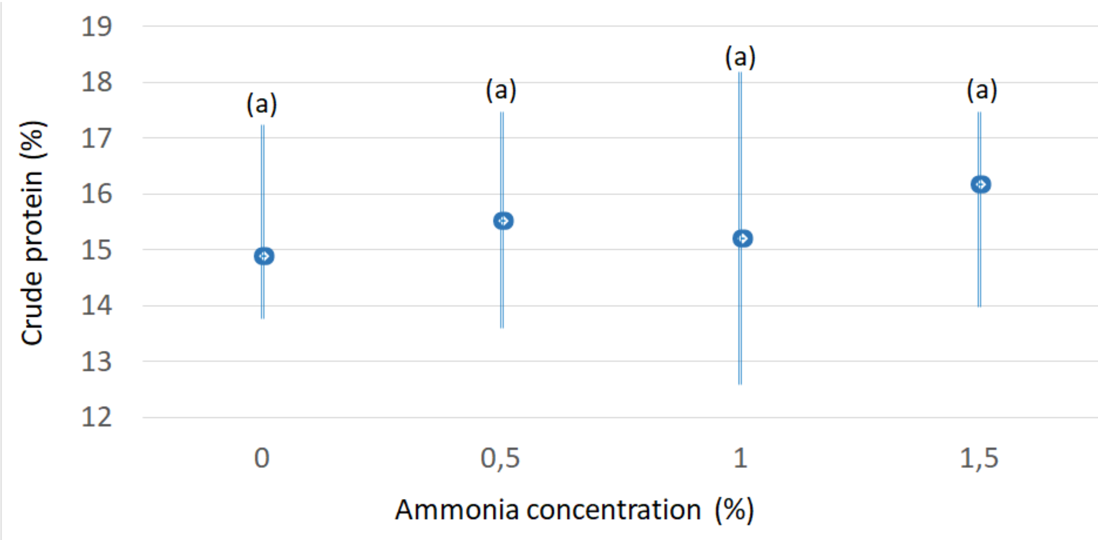


[a,b] The values differ from each other ($p<0.05$). Means were compared using the Tukey's test.
Figure 1. Effect of ammonia gas on deoxynivalenol reduction in wheat samples.

The hypothesis that different concentrations of ammonia gas could be efficient in eliminating deoxynivalenol in wheat grains was confirmed. Regarding deoxynivalenol deactivation by ammonia gas, the results obtained corroborate the studies by Dias (2018), Allameh *et al*. (2005) and Borràs-Valllverdú *et al*. (2020), who reported that ammonization is the most effective process to decontaminate animal feed.

Samarajeewa (1990) and Carão *et al*. (2014) highlighted reduction above 93% in contamination levels with concentrations ranging from 0.5 to 5.0% of ammonia on different substrates. In the treatment of wheat with ammonia steam for two hours at 90ºC, Borràs-Vallverdú *et al* (2020) observed a reduction of more than 75% of DON.

In experiment 2 (Fig. 2) shows that the amount of protein did not differ statistically between the control and wheat samples treated with different concentrations of ammonia.



[a]The values do not differ from each other ($p<0.05$). Means were compared using the Tukey’s test.

Figure 2. Protein (%) in whole wheat samples treated with different concentrations of ammonia.

(a)

The results found agree with Brekke (1977). These authors found that the ammonization process does not reduce the nutritional value of corn. On the contrary, they observed that trout fed corn treated with ammonia performed better than those fed untreated corn.

In experiment 3 (Table 1) it demonstrates the hematological profile of rats fed with grains treated with ammonia.

In the test with Wistar rats (*Rattus norvegicus*), the animals did not reject the feed made with treated wheat. In contrast, they preferred the ammonia-treated chow to the basal diet.

The parameters of the hematological examination (Table 1) did not show significant changes, except for triglycerides, demonstrating that ammonia was not toxic and that the compounds formed by the breakdown of DON were also not harmful to the health of rodents. These findings corroborate the studies by Brekke *et al*. (1979) and Allameh *et al*. (2005) where they reported that broiler chickens and pigs had good acceptance for the consumption of cereals treated with ammonia.

In the test with Wistar rats (*Rattus norvegicus*), the animals did not reject the feed made with treated wheat. In contrast, they preferred the ammonia-treated chow to the basal diet.

Table 1. Hematological evaluation of rats fed with wheat treated with ammonia at different concentrations

Conc. Ref. Val.	ALT (32 a 83)	AP (41 a 959)	Creatin. (0.24 a 1.20)	Urea (26 a 58)	Cholest. (45 a 76)	TG (22 a 100)	PT (4 a 6.9)	Album. (2 a 3.5)	Globul. (2.1 a 5.4)
BD	45.04[a]	582.28[a]	0.77[b]	53.60[a]	58.83[a]	128.85[b]	6.35[a]	2.99[a]	3.35[a]
BD+WC	48.62[a]	567.45[a]	1.00[a]	39.33[bc]	69.87[a]	182.16[a]	6.69[a]	3.27[a]	3.41[a]
BD+0.5%	45.19[a]	510.43[a]	0.68[b]	38.17[c]	65.92[a]	52.47[c]	6.52[a]	3.36[a]	3.16[a]
BD+1.0%	42.94[a]	558.13[a]	0.80[b]	46.36[ab]	64.58[a]	53.78[c]	6.60[a]	3.21[a]	3.38[a]
BD+1.5%	43.62[a]	548.76[a]	0.76[b]	43.77[bc]	65.43[a]	77.98[c]	6.17[a]	3.28[a]	2.88[a]

[a.b.c.d] The means followed by the same lowercase letters in the columns do not differ from each other ($p < 0.05$) by the Bonferroni test. Means were compared by the Tukey's test. (Ref. Val - reference values), (ALT- alanine amino-transferase), (AP- alkaline phosphatase), (BD- basal diet), (WC- wheat control), (Conc.- concentration), (Creatin.- Creatinine), (Colest.- Cholesterol), (TG.- triglycerides), (TP- Total Protein), (Album.- Albumin), (Globul.- Globulin).

The parameters of the hematological examination (Table 1) did not show significant changes, except for triglycerides, demonstrating that ammonia was not toxic and that the compounds formed by the breakdown of DON were also not harmful to the health of rodents. These findings corroborate the studies by Brekke *et al*. (1979) & Allameh *et al*. (2005) where they reported that broiler chickens and pigs had good acceptance for the consumption of cereals treated with ammonia.

The triglyceride levels found in the groups receiving the basal diet and untreated wheat were above normal parameters. Normal levels of triglycerides in male Wistar rats vary, respectively, from 22 to 100mg/dL (Santos *et al*, 2010). The composition of the basal diet has a higher amount of fat compared to wheat treated with ammonia. It may also be associated with the presence of deoxynivalenol in wheat, which could be causing changes in liver function.

The results obtained in the histopathological examination (liver and kidneys) demonstrate that the feeding of rats with wheat treated with ammonia did not interfere in these parameters. Neal *et al*. (2001) also did not observe any lesions associated with hepatocarcinogenesis after feeding peanut flour treated with ammonia to rats.

Exposure to DON poses serious health risks to animals. According to Dias (2018), chronic exposure to low levels leads to reductions in skin temperature and blood plasma globulins. Freire *et al.* (2007) and Munkvold *et al*. (2019) mentioned that DON ingested in small doses by pigs and other animals can cause weight loss and feed refusal. The animals were weighed every three days in our experiment and all groups had a gradual weight gain. It was found that treatment of wheat kernels with ammonia deactivated DON without compromising crude protein composition and animal health. Therefore, the ammonization of wheat will allow a more efficient use of this cereal in animal feed, avoiding the losses caused by the consumption of DON.

New studies must be carried out to evaluate the effect of ammonia gas on wheat samples artificially contaminated with different concentrations of deoxynivalenol, to standardize the technique. Also verify in the animals, when they consume high concentrations of deoxynivalenol, hematological and histopathological alterations in the liver, kidneys, and intestine, which could not be verified in this experiment, since the wheat used as a positive control did not present high concentrations of this mycotoxin.

CONCLUSION

The treatment of wheat grains with ammonia gas at a concentration of 1 to 1.5% was efficient in deactivating deoxynivalenol, did not influence the protein composition of wheat and proved to be safe for feeding *Rattus norvegicus.*

ACKNOWLEDGMENTS

To the University of Passo Fundo, Aveclean and Biotrigo Genetica for the financial support in carrying out this research.

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