Jurnal Kajian Veteriner
ISSN: 2356-4113
E-ISSN: 2528-6021
Vol. 11 No. 1:86-92 (2023)
DOI:https://doi.org/10.35508/jkv.v11i1.11011

Starch and Aflatoxin B1 Content of Corn Grain with Different Water Content Added with Synthetic Mold Inhibitor

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ABSTRAK

Penggunaan jagung sebagai pakan harus memenuhi standar kualitas kimia yang dipersyaratkan untuk menjamin keamanan pakan. Penggunaan penghambat jamur sintetis merupakan salah satu strategi untuk menghambat pertumbuhan jamur dan produksi metabolit sekunder yang dapat menurunkan kualitas kimia jagung. Penelitian ini bertujuan untuk menganalisis kandungan amilum (pati) dan aflatoksin B1 (AFB1) jagung kuning pipilan yang terdiri dari tiga level kadar air dan disuplementasi produk anti jamur sintetis. Sejumlah 750 kg jagung kuning pipilan digunakan dalam penelitian ini. Penghambat jamur (PJ) komersial yang digunakan diperoleh dari PT Japfa Comfeed Tbk, Indonesia dengan dosis 450 g/ton jagung. Percobaan dua faktor (Level kadar air: 13, 14 dan 15%; penghambat jamur PJ: -, +) digunakan pada penelitian ini. Hasil uji ANOVA membuktikan bahwa kecuali kandungan pati, interaksi yang signifikan antara LKA dan PJ terhadap kandungan aflatoksin B1 jagung yang disimpan selama 60 hari. Jagung (LKA berbeda dan tanpa PJ) memiliki kandungan AFB1 lebih tinggi dari yang diberi PJ. Simpulannya, penggunaan penghambat jamur efektif menghambat pertumbuhan jamur sehingga produksi aflatoksin B1 dapat ditekan. Penggunaan PJ sintetis tidak mempengaruhi kandungan pati jagung biji selama 60 hari penyimpanan.

Kata kunci : aflatoksin; amilum; keamanan pakan; penghambat jamur

INTRODUCTION

According to Indonesia National Standard Board (2013), the quality of corn as feed must meet the quality requirements with a maximum aflatoxin content of <100 ppb. Generally, moisture content (free and bound) is greatly related to the aflatoxin content of corn grains. Li *et al.* (2021) and Zhu *et al.* (2023) stated that newly harvested corn grains had high water content around 18-40%. Thus, it is essential for that corn to be dried immediately under the sun or by using the drying machine (vertical or horizontal drier) to achieve the low moisture content (<14%) in order to prevent the contamination of toxic fungus. *Aspergillus flavus* was the dominant fungi that contaminated corn, followed by Fusarium which produced Deoxynivalenol, Zearalenon, and Fumonisin (Tangendjaya *et al.*, 2008). The propagation of the *Aspergillus flavus* depend on temperature, pH, and humidity. According to the published

research (Hassane *et al.*, 2017; Lahoura *et al.*, 2016), supporting condition for *Aspergillus flavus* to produce toxin was in thermal range between 25 and 30 °C, 80% relative humidity, water activity of 0.95-0.99, aerobic atmospheric conditions, and a moisture

Besides lipids, two nutrients that exceedingly increased the multiplication of *Aspergillus flavus* and *Aspergillus flavus* toxin quantity were arginine and stachyose (Liu *et al.*, 2016). The findings of Liu *et al.*, (2016) demonstrated that *Aspergillus flavus* will decrease the nutrient content of grains to aflatoxins.

Aflatoxin, a fungal poison, is produced by the Aspergillus spp. during their investment and growth in cereal and legume seeds (Ting *et al.*, 2020; Shabeer *et al.*, 2022). Aflatoxins can cause cirrhosis and liver cancer in human beings and animals (Navale *et al.*, 2021). The severity of aflatoxin toxicity in each livestock is different depending on the level of aflatoxin. Ducling and Turkey were the most vulnerable type of livestock to the danger of aflatoxin (Diaz *et al.*, 2008). Poultry exposed to aflatoxin may not experience death but experience a decrease in productivity, causing large economic losses.

To impede the spreading out of pathogenic fungi and aflatoxin improvement, it can be done by reducing the corn grain's water content either using drying machine or sun drying, supplementing corn grains with Mintai Feed Anti-mold (Nalle *et al.*, 2023), or using ozone gas (Hidayah *et al.*, 2021). Supplementation of 450 g/ton a commercial mold inhibitor containing propionic acid, arsenic, and lead was efficacious in prolonging the quality of corn seeds with low moisture content (<12%) but did not reduce the content of aflatoxin B1 and starch (Nalle *et al.*, 2022a,b). The issue of food and feed safety is still big and important issue in the world, so intensive and comprehensive research about sustaining corn quality using feed additives (mold inhibitors) needs to be evaluated. The present research was done to assess the chemical properties (AFB1 and starch) of corn that was stored using a synthetic mold inhibitor.

MATERIALS DAN METHODS

Corn Grains and Mold Inhibitor Product

The yellow corn grains used in the present study had three different levels of water content which were 13, 14, and 15%. The aflatoxin content of corn was <50 ppb. These grains were purchased from the local corn distributor in the Kupang area, West Timor, Indonesia. A commercial fungus inhibitor was obtained from feed industry. The active compound of this mold inhibitor product was propionic acid (570 g/kg), lead (30 ppm), and arsenic (0.54 ppm). The dose of mold

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inhibitor product implemented was at the level of the factory recommendation (0.45%).

Experimental Design

The present study was implemented factorial experiment design with two factors. Water Content (WC; 13%, 14%, and 15%) was set as the first major factor. Mold inhibitors (-, +) was placed as the second factor. Thus, the total treatment combination was six with five replications containing 20 kg of corn. The corn grains were stored in room temperature (29.5 – 34.3 °C) with the relative humidity (58-74% in the morning; 45-62% during the day time, and 55-81% in the evening).

Sampling Procedure

A sampling process of corn was done at the end of the experiment (day 60) using the procedure of sampling described by Nalle *et al.* (2022a). After that, the reduction of corn samples was performed using seed sampler equipment (Retch) to produce a representative sample that was ready for laboratory analysis.

Chemical Analysis

Starch analysis was conducted using the Luff Schroll method (SNI 01-2891-1992) at SIG Saraswanti Laboratory Bogor.

Aflatoxin B1 quantitative assay was carried out at SEAMEO BIOTROP Laboratory, Bogor-Indonesia with a Chromatography apparatus (Thin-layer chromatography, TLC) (Bainton *et al.*, 1980).

Analysis of Statistic

Online SAS software was used to evaluate the quantitative data. The significant between the treatments was further examined using the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Table 1 depicted the amount of Aflatoxin B1 (AFB1) and starch of corn at the end of the experiment. Statistical analysis showed that WC x MI had no influence ($P>0.05$) on the corn starch quantity along the trial, similar to Nalle *et al.* (2022b). Conversely, WC and MI treatment combination greatly changed ($P<0.001$) the AFB1 quantity of corn grains during the experimentation.

The AFB1 content of corn grains with 13 and 15% WC with MI addition was lower ($P<0.05$) than those without MI. The corn with 14% WC added with MI had similar ($P>0.05$) AFB1 content to those without MI. However, it seems that the AFB1 content of 14% WC corn added with MI was lower than those which were not added with MI. This indication of the present result was that the MI product used in the present study worked well to inhibit the growth of toxic fungus to produce secondary metabolites. However, the

reduction of AFB1 in shelled corn with 14 and 15% WC added with MI still exceeds the aflatoxin standard of corn regulated by the Indonesian National Standard (2013). According to Indonesia National Standard Board (2013) the maximum content of aflatoxin of first-quality corn as feed was 100 ppb and for second-quality was 150 ppb. This showed that the addition of MI in the corn grains with a WC of 14 to 15% which had been contaminated with aflatoxins was not valuable.

Table 1. The Average Content of Starch and Aflatoxin B1 (AFB1) as Influenced by the Treatment Combination

Water Content	Mold Inhibitor	Starch	AFB1
		(% as fed)	(ppb)
13%	-	59.4	307^{bc}
	+	59.4	19.5^{d}
14%	-	57.2	6676^{a}
	+	57.1	6633^{a}
15%	-	61.1	4133^{b}
	+	60.0	237^{c}
Std. Err. Mean		1.628	73.4
Main factors			
WC			
13%		59.4	163.5^{c}
14%		57.2	6655^{a}
15%		60.5	2185^{b}
Std. Err. Mean		1.151	52.4
MI	-	59.2	3706^{a}
	+	58.9	2295^{b}
Std. Err. Mean		0.940	43.0
Significance			
WC		P>0.05	P<0.001
MI		P>0.05	P<0.001
WC × MI		P>0.05	P<0.001

Notes: Significant difference (P<0.05) was determined by the difference of superscript in the same column; Std. Err. Mean = Standard Error of Mean. AFB1 detection limit = 3.01 ppb

The low AFB1 content of the corn added with MI during the experimental period occurred by several mechanisms. Josan and Sek-Yeo (2010) explained that there were several mechanisms involved in inhibiting toxin fungal growth namely the reduction of pH, disruption the bacteria cell membrane, metabolic energy, and the anion impact.

The current findings were dissimilar from what was reported by Nalle *et al.,* (2022). The differences may occur due to different moisture levels. The moisture content level of

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corn used in the study by Nalle *et al.* (2022) was <12%, while the present research used 12 to 15% WC.

Regarding the first main effect (WC), the AFB1 level of corn with a moisture content of 14% was higher (P<0.05) than those of 13 and 15% WC, this was an unacceptable result. The high AFB1 level of 14% WC corn might be due to the optimum condition of *Aspergillus flavus* to grow and produce AFB1. According to previous researchers, factors such as pH, light, water content, water activity, and temperature during the storage substantially altered the development of *Aspergillus flavus* and aflatoxin configuration (Mulyati *et al.,* 2021; Priesterjan *et al.,* 2020).

Regarding the second factor, corn added with MI had lower (P<0.05) AFB1 than the control treatment. Even though the addition of MI decreased the B1-type aflatoxin level, this level was still too high. This AFB1 level was far beyond the level recommended by Indonesia National Standard Board (2013). Thus, corn harvested at the end of this experiment cannot be used anymore as poultry feed. The result of this experiment indicated that the synthetic MI used in the present study did not work well to stabilize the mycotoxin content (Table 1). Thus, this commercial mold inhibitor was not effective to be used in corn that has been already infected by aflatoxin. Nalle *et al.* (2022) claimed that the same MI succeeded to maintain the primary AFB1 amount of corn when using fresh corn.

CONCLUSION

To sum up, the high water content of corn grains has a big effect on the enhancement of corn's AFB1. The use of synthetic mold inhibitors effectively inhibits the growth of toxic fungi so that the production of AFB1 can be depressed. The use of synthetic mold inhibitors did not effectively change the content of corn starch during storage.

ACKNOWLEDGEMENTS

Gratefully thank you to the Polytechnic of Agriculture Kupang for the research grant (Contract: 01/P3M/SPDIPA.023.18.2.677616/2022/DIPA.023.18.2.677616/2022). We also would like to thank you for the in-kind contribution provided by the stakeholder (PT Japfa Comfeed) for the product of Anti-mold, and for Ariska Benu and Yafet for their assistance during the research.

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