Seasonal Variations In Aflatoxin B_1 Levels In Corn And Wheat: An Observational Study

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Abstract
Aflatoxin B_1 is considered the most toxic aflatoxin, produced by fungi of the genus *Aspergillus*, which can contaminate corn and wheat, especially under conditions of high humidity and temperature, leading to harmful effects on both human and animal health. To ensure a safe final product, aflatoxin B_1 levels in food and feed are strictly regulated by international standards. For instance, the European Union sets maximum limits of 5 ppb for AFB_1 and 20 ppb for total aflatoxins in animal feed. Therefore, continuous monitoring is essential to protect public health and maintain agricultural market stability.This study aimed to analyse the concentration of aflatoxin B_1 in corn and wheat samples collected from a farm in northeastern Romania. A total of thirty feed samples (seven wheat and twenty-three corn) were examined over two distinct periods autumn-winter and spring-summer. Statistical analysis revealed significant differences between the two types of feed ($P < 0.05$), as well as between the two seasons ($P < 0.001$). The average aflatoxin B_1 concentration was 4.788 ppb during the autumn-winter season and 4.157 ppb in the spring-summer season, with a mean seasonal difference of 0.63 ppb. These findings confirm that aflatoxin B_1 levels vary depending on both feed type and seasonal conditions.

Keywords: aflatoxin B_1, corn, statistical analysis, seasonal variation, wheat.

1. Introduction

Cereals are essential for both human and animal nutrition and are widely used in the food industry and as components of animal feed [1]. However, they are susceptible to contamination with mycotoxins produced by fungi, among which aflatoxins represent a major concern [2]. These compounds are highly toxic, mutagenic, and carcinogenic, with aflatoxin B_1 (AFB_1) being the most dangerous. AFB_1 is produced primarily by fungi of the genus *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus* [3]. It is a stable compound that resists conventional food processing, making it difficult to eliminate and leading to significant economic losses [4]. Contamination with AFB_1 is particularly common in corn and wheat, two of the most widely cultivated cereal crops. Corn is especially vulnerable due to its susceptibility to high humidity and elevated temperatures during storage, which promote fungal growth [5]. Aflatoxins compromise not only the quality and safety of agricultural products, but also pose serious health risks to consumers. Aflatoxin B_1 is a well-established human carcinogen, strongly associated with liver cancer and other hepatic diseases [6]. Although food processing methods may reduce aflatoxin levels to some extent, AFB_1 is highly resistant and often remains in contaminated products [7]. Moreover, mycotoxins present a dual threat-affecting both human and animal health, as they can be transferred through contaminated feed. It is estimated that approximately 25% of the global cereal harvest is

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affected by mycotoxins annually [8]. To prevent AFB_1 contamination, it is crucial to implement good agricultural practices, such as proper drying and storage of grains under optimal conditions to limit fungal proliferation [9,10]. In addition, strict monitoring and regulatory control of aflatoxin levels in food and feed are essential to safeguard public health and maintain the stability of the agricultural economy [11].

2. Materials and methods

The data collected for this observational study aimed to analyze the aflatoxin B_1 content in 30 feed samples. Two different types of feed were considered: corn and wheat. A total of 23 corn samples and 7 wheat samples were collected from a farm in northeastern Romania. The samples were gathered throughout the year 2023, and the aflatoxin B_1 content was assessed in two distinct seasons: autumn-winter and spring-summer. The analysis of aflatoxin B_1 in the feed was performed using the ELISA method, a quantitative technique based on the antigen-antibody reaction. The presence of aflatoxin B_1 was detected through a colorimetric reaction, with the intensity measured using an ELISA immunoenzymatic reader. The procedure involved several steps: adding a conjugated solution to dilution wells, followed by sample addition and mixing. The mixture was then transferred to antibody-coated microwells for incubation. After washing the wells, a substrate solution was added, leading to color development inversely proportional to the mycotoxin concentration. A stop solution was subsequently applied, altering the color, and absorbance was measured at 450 nm. The analysis was conducted using Romer Labs kits, following EN ISO 9001 and EN ISO 17025 standards, according to the manufacturer's protocol for the AgraQuant kit [12]. After compiling the database, several statistical parameters were calculated, including the median, mean, standard deviation, standard error of the mean, confidence interval of the mean, minimum, and maximum values. Statistical hypotheses were formulated, a significance threshold (α = 0.05) was selected, and the difference in aflatoxin B_1 levels between the autumn-winter and spring-summer seasons was used to determine the appropriate type of Student's t-test.

3. Results and discussion

Table 1 presents the aflatoxin B_1 content recorded in the 30 analyzed feed samples. According to the calculated statistical parameters, the average content during the autumn-winter season was 4.788 ppb, while in the spring-summer season it was 4.157 ppb.

Table 1. Aflatoxin B_1 content parameters recorded for 30 feed samples

Nr. Crt.	Feed sample	Aflatoxin B_1 (ppb)		Seasonal difference (ppb)
		Autumn-Winter	Spring-Summer	
1	Corn	4.70	4.06	0.64
2	Corn	4.85	2.44	2.41
3	Corn	5.38	4.03	1.35
4	Corn	4.70	4.24	0.46
5	Corn	3.79	3.63	0.16
6	Corn	6.55	4.96	1.59
7	Corn	4.38	4.27	0.11
8	Corn	5.46	4.25	1.21
9	Corn	4.55	3.70	0.85
10	Corn	3.64	3.01	0.63
11	Corn	4.69	4.45	0.24
12	Corn	5.34	4.80	0.54
13	Corn	4.51	4.30	0.21
14	Corn	4.96	3.80	1.16
15	Corn	4.33	4.22	0.11
16	Corn	4.78	4.70	0.08
17	Corn	4.31	4.20	0.11
18	Corn	5.16	3.90	1.26
19	Corn	4.72	3.90	0.82
20	Corn	3.76	3.35	0.41
21	Corn	5.69	4.80	0.89
22	Corn	5.70	5.00	0.70
23	Corn	4.98	4.22	0.76
24	Wheat	4.31	4.12	0.19
25	Wheat	4.55	4.23	0.32
26	Wheat	4.7	4.46	0.24
27	Wheat	4.85	4.79	0.06
28	Wheat	4.8	3.95	0.85
29	Wheat	4.22	4.17	0.05
30	Wheat	5.29	4.78	0.51
Median		4.71	4.22	0.525
Average		4.788	4.157	0.630
Standard deviation		0.618	0.564	0.548
Standard error		0.225	0.206	0.200
Average confidence limit		1.787	1.787	1.787
Minimum value		3.64	2.44	0.05
Maximum value		6.55	5	2.41

The mean seasonal difference of 0.63 ppb indicates a variation in aflatoxin B_1 levels between the two seasons across both feed types.

To determine whether to apply a Student's T-test with equal or unequal variances, an F-test was first conducted (Table 2). The P-value obtained was 0.0418, which is less than the significance level of 0.05, indicating that the variances between the two sample groups (corn and wheat) are significantly different. As a result, the Student's T-test assuming unequal variances was applied. In Table 3, the T-test assuming unequal variances revealed a P-value of 0.0191 (two-tailed), which is also less than 0.05. This result indicates that the difference between the two feed types is statistically significant. Therefore, the null hypothesis was rejected in favor of the alternative hypothesis, suggesting that the aflatoxin B_1 content differs significantly between corn and wheat samples.

Table 2. F-Test Two-Sample for Variances

	Variable 1 (Corn)	**Variable 2 (Wheat)**
Mean	0.726086957	0.317142857
Variance	0.333306719	0.08012381
Observations	23	7
df	22	6
F	4.159896058	
P (F<=f) one-tail	**0.041833406**	**P = 0.041833406 < 0.05, indicating that the Student's T-test is applied with unequal variances.**
F Critical one-tail	3.856403079	

Table 3. t-Test: Two-Sample Assuming Unequal Variances

	Variable 1 (Corn)	**Variable 2 (Wheat)**
Mean	0.726086957	0.317142857
Variance	0.333306719	0.08012381
Observations	23	7
Hypothesized Mean Difference	0	
df	21	
t Stat	2.539200045	
P(T<=t) one-tail	0.000954328	
t Critical one-tail	1.720742903	
P(T<=t) two-tail	**0.019086559**	**P = 0.019086559 < 0.05, indicating statistically significant differences between the two samples, leading to the acceptance of the alternative hypothesis.**
t Critical two-tail	2.079613845	

Table 4. t-Test Paired Two Sample for Means

	Variable 1 (Autumn-Winter)	Variable 2 (Spring-Summer)
Mean	4.778333333	4.157666667
Variance	0.381931609	0.31857023
Observations	30	30
Pearson Correlation	0.573545811	
Hypothesized Mean Difference	0	
df	29	
t Stat	6.302688294	
P(T<=t) one-tail	3.48327E-07	
t Critical one-tail	1.699127027	
P(T<=t) two-tail	**6.96653E-07**	**The P-value is very small, < 0.001, indicating that the differences between the two seasons autumn-winter and spring-summer are statistically significant.**
t Critical two-tail	2.045229642	

To further validate the findings, a paired two-sample T-test for means was applied to compare the seasonal variation in aflatoxin B_1 levels (Table 4). The test yielded a very small P-value of 6.97×10^{-7}, which is well below the 0.001 threshold. This confirms that there are highly significant differences in aflatoxin B_1 content between the autumn-winter and spring-summer seasons.

4. Conclusions

The statistical analysis revealed significant differences between the two types of feed (corn and wheat), as well as between the aflatoxin B_1 content recorded in the autumn-winter and spring-summer seasons. The P-value, which was lower than 0.0001, confirms a high degree of variability in the data across seasons. These findings suggest that aflatoxin B_1 levels are influenced by both the type of feed and seasonal conditions, highlighting the importance of regular monitoring to ensure feed safety.

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