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PREDICTION OF AFLATOXIN RISK IN CORN FEED BASED ON PHYSICAL QUALITY PARAMETERS: DEVELOPMENT AND VALIDATION OF ORDINAL MODELS

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ABSTRACT

Aflatoxin contamination in feed corn is a critical concern, especially in tropical regions where post-harvest moisture and storage conditions support fungal growth. This study developed and validated an ordinal logistic regression model to predict the risk of aflatoxin contamination using corn physical quality parameters. A total of 384 valid observations were used for model development. Aflatoxin was classified into three ordinal risk categories, while moisture content, damaged kernels, broken kernels, moldy kernels, and foreign materials were classified into ordinal quality classes based on adjusted feed-corn quality criteria. The initial model included all physical quality parameters, and predictor selection was performed using Wald statistics. The final model retained moisture content, broken kernels, and moldy kernels as significant predictors, whereas damaged kernels and foreign materials were excluded. Model fitting indicated that the final model significantly improved prediction compared with the intercept-only model ($P<0.001$). Goodness-of-fit statistics were non-significant, and the Nagelkerke pseudo-R^2 reached 0.519, indicating adequate explanatory capability. Model validation with 105 independent samples yielded an overall classification accuracy of 73.33%, with high class-specific accuracy in the low-risk and high-risk categories. The moderate Cohen's Kappa value ($\kappa = 0.597$) indicated meaningful agreement between actual and predicted risk classes beyond chance. Progressive validation across increasing sample sizes ($N = 10–200$) showed stable performance, with accuracy ranging from 74.50 to 83.20% and Kappa values approaching a stable moderate-agreement range. These findings suggest that ordinal logistic regression can serve as an early-warning tool for screening aflatoxin risk in feed corn based on physical quality indicators.

Keywords: Aflatoxin, Feed corn, Ordinal logistic regression, Physical quality, Risk prediction, Model validation.

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1. INTRODUCTION

Corn aflatoxin contamination is a major problem in the poultry feed industry, given its significant impact on livestock health, productivity, and food safety. Aflatoxin, produced by molds of the genus *Aspergillus*, is known to be toxic and carcinogenic, and to impair liver and immune system function in livestock (Shabeer et al., 2022). Among aflatoxigenic fungi, *Aspergillus flavus* is the predominant species infecting maize and the principal producer of aflatoxin B, whereas *A. parasiticus* is also of major concern because it produces both B- and G-type aflatoxins (Poungpong et al., 2024; Gao et al., 2025; Bailly et al., 2025). Environmental stresses such as drought, high temperatures, climatic variability, seasonal fluctuations, and insect damage increase maize susceptibility to *Aspergillus* infection and aflatoxin contamination (Riungu et al., 2024; Nji et al., 2025). Infection by *A. flavus*

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deteriorates maize quality by reducing its nutritional value and storage stability while increasing aflatoxin accumulation (Manzoor et al., 2026). Consequently, aflatoxin-contaminated maize has become a major feed-safety concern because it impairs animal performance, allows toxin carry-over into milk and other animal-derived products, and poses significant risks to public health and international trade (Pokoo-Aikins et al., 2024; Akullo et al., 2025; Sharifi et al., 2026). Exposure to aflatoxin in poultry can decrease growth, egg and meat productivity, feed conversion efficiency, immune response and increase susceptibility to disease and mortality (Fouad et al., 2019; Mahato et al., 2019). The risk of contamination is increasingly high in tropical regions with temperature and humidity conducive to mold growth, so early detection and control are critical in the feed raw material supply chain. Aflatoxin contamination in corn is one of the main problems in the poultry feed industry, considering that corn is the main raw material for energy sources in poultry rations and is a commodity that is susceptible to aflatoxin contamination (Mahato et al., 2019; Dövényi-Nagy et al., 2020).

The identification and quantification of aflatoxin in food and feed are generally performed using laboratory analytical methods, e.g., thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), and enzyme-linked immunosorbent assay (ELISA). The method has good sensitivity and accuracy, especially for confirming aflatoxin levels, but in practice, they require sample preparation stages, special instruments, analysis time, and skilled labor, making it less ideal for rapid and routine testing on an industrial scale (Mahato et al., 2019; Zhang & Banerjee, 2020). This practical gap has spurred the development of rapid, portable screening technologies, including fluorescence-based systems and handheld spectrometers for aflatoxin detection and sorting in corn (Smeesters et al., 2023; Yao et al., 2023). Even so, feed industries still require a simple decision-support layer that can be applied before confirmatory testing. Physical quality parameters of incoming corn may therefore serve as an operational proxy for early risk screening, particularly when rapid decisions are needed for drying, sorting, segregation, or further aflatoxin testing.

One practical approach for an initial screening system is to use corn physical quality parameters as an indirect indicator (Proxy) for risk of aflatoxin contamination. Parameters such as moisture content, damaged kernels, broken kernels, moldy kernels, and foreign materials have long been used in corn quality assessment, including in the Indonesian National Standard (SNI) 8926:2020. Biologically, these parameters are relevant because they reflect postharvest handling, drying, sorting, transport, and storage conditions that may influence mold development and mycotoxin risk (Domenico et al., 2015; Suleiman et al., 2018; Lu et al., 2024). However, the relationship between physical qualities and aflatoxin contamination is not always linear or single, as aflatoxin formation is affected by the interaction of various factors, including moisture, temperature, storage time, kernel damage, the presence of toxigenic fungi, as well as microenvironmental conditions during storage (Lu et al., 2024; Marín et al., 2024). These considerations place physical quality parameters as early risk indicators for screening and decision support, rather than as substitutes for quantitative aflatoxin analysis. Evidence from corn storage and postharvest studies shows that moisture content, damaged kernels, and broken kernels are associated with quality deterioration, fungal growth, and mycotoxin risk (Suleiman et al., 2018; Shabeer et al., 2022; Matusse et al., 2025). In addition, path analysis of stored corn has shown that physical, chemical, and microbiological parameters may influence the occurrence of aflatoxin both directly and indirectly (Domenico et al., 2015).

Recent studies have developed predictive models for aflatoxin contamination in corn using machine learning, Bayesian networks, mechanistic models, meteorological data, satellite-derived vegetation indices, and soil properties (Liu et al., 2021; Castano-Duque et al., 2022; Branstad-Spates et al., 2023). These approaches are valuable for early warning at regional or crop-production scales, yet they are less directly aligned with the operational decision point faced by feed industries during raw material reception. At this stage, risk classification often relies on threshold-based decisions, although aflatoxin risk may occur across overlapping physical quality conditions rather than in clearly separated categories. This creates a need for a model that can preserve the ordered structure of risk classes while still providing probabilistic information. Ordinal logistic regression is appropriate for this purpose because it is designed for ordered categorical response variables and, through the cumulative logit or proportional odds framework, estimates both predictor effects and cumulative probabilities for each response level (Agresti, 2010; Harrell, 2015). In this study, the approach allows aflatoxin risk to be expressed not only as a final class assignment but also as a probability profile across low, moderate, and high-risk categories.

This study aims to develop an ordinal logistic regression model to predict the risk of aflatoxin contamination in raw corn materials based on physical quality parameters. The model estimates cumulative probabilities for ordered risk categories, allowing aflatoxin risk to be interpreted as a probability profile rather than a fixed class assignment. Progressive validation across different sample sizes was also used to assess the model's stability and generalization. By using routinely measured quality parameters at the feed-mill receiving point, this study positions physical quality assessment as a practical layer of early risk screening. The proposed model is intended to support faster decision-making on whether incoming corn lots require drying, sorting, segregation, rejection, or confirmatory aflatoxin testing.

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2. MATERIALS AND METHODS

2.1. Research Design

This study used a quantitative analytical approach to develop a model to predict the risk of aflatoxin contamination in corn, a raw material for the feed industry. The model is built based on the physical quality parameters of corn, namely moisture content (MC), damaged kernels (BK), broken kernels (DK), moldy kernels (MK), and foreign materials (FM). The response variable in this study was the aflatoxin (AFL) risk level, which was classified ordinally, while the predictor variable was the physical quality class of corn. The research flow was arranged in stages, starting from corn sampling, measurement of physical quality parameters and aflatoxin levels, data cleaning, ordinal classification of variables, development of ordinal logistic regression models, selection of predictors, and validation of model performance. In general, the stages of model development and validation are presented in Fig. 1.

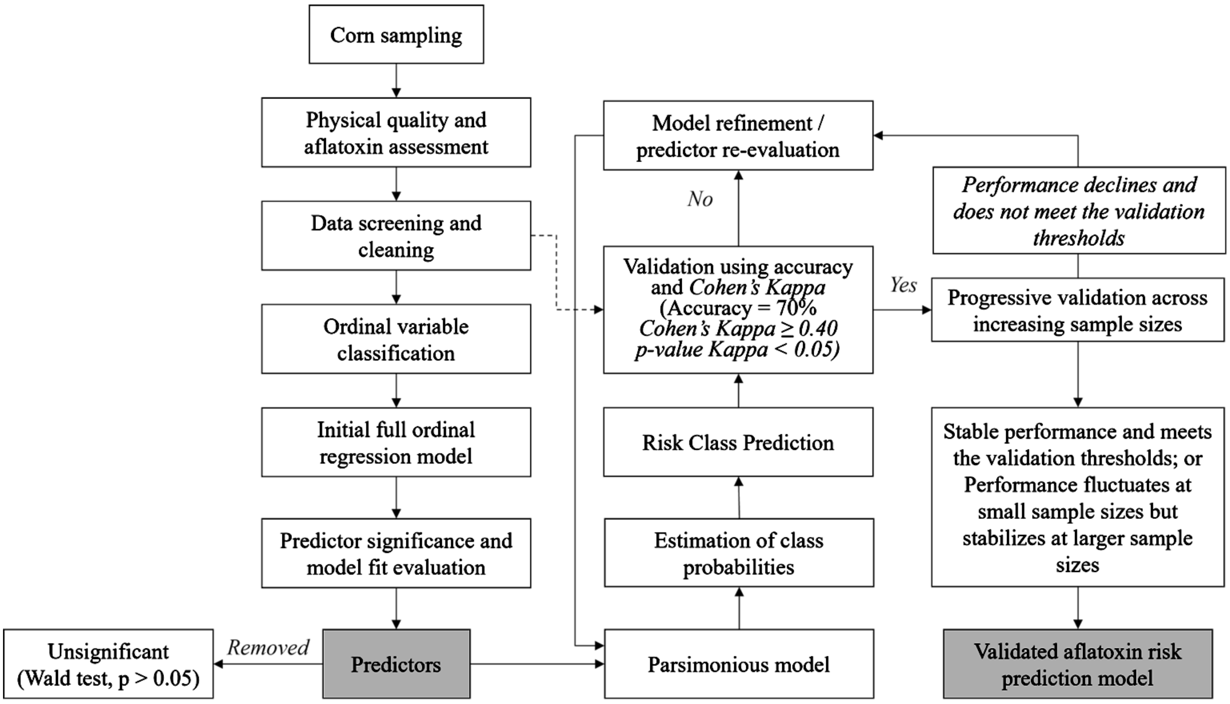

Fig. 1: Analytical workflow for developing and validating an ordinal logistic regression model for aflatoxin risk prediction in feed corn.

2.2. Sampling

The research data were obtained from observations of the physical quality of corn pipilan and the aflatoxin contamination levels in corn samples used as raw materials for the feed industry. Sampling was carried out in the raw material reception area on corn transport vehicles that have just arrived at the factory site, prior to dismantling and storage. In each transport vehicle, samples were taken from three parts of the corn pile in the vehicle's tub/dump: the top, middle, and bottom. From each part, ± 250 g were sampled, so that a total initial sample of about ± 750 g per vehicle was obtained. The samples from the three points were then combined and homogenized into a composite sample representing a single raw material acceptance unit.

The dataset used to develop the model consisted of 390 corn samples. After checking the completeness of the data on the response variables and predictors, a total of 384 valid observations were used in the ordinal logistic regression modeling. In addition to the model development dataset, this study also uses additional datasets for prediction validation. The validation dataset consists of 150 additional samples for initial evaluation of model performance, and 200 additional samples for progressive validation. All validation samples were obtained using the same sampling procedure: from corn transport vehicles that had just arrived at the factory's raw material reception area.

The validation dataset is not used to estimate the model's coefficients, but is used to test the model's predictive ability on new data. At the validation stage, the actual aflatoxin risk class was compared with the model's predicted risk class and evaluated using the confusion matrix, classification accuracy, and Cohen's Kappa. Specifically for phased validation, testing is performed on progressively increasing sample sizes to assess the stability of model performance.

2.3. Measured Parameters

2.3.1. Moisture Content: The moisture content of corn was measured using *a grain moisture tester*. Before measurement, the appliance is switched on and adjusted to the code or measurement mode for the corn commodity. The corn sample is then placed in the measuring chamber to the tool's capacity, and the tool automatically reads the moisture content and displays the result as a percentage (%). To improve the reliability of the results, moisture content measurements are repeated, and the average is used as the final moisture content for each sample.

2.3.2. Physical Quality: The assessment of the physical quality of corn was carried out by referring to the quality testing procedure for feed corn as used in the Indonesian National Standard SNI 8926:2020. Corn samples were weighed up to 100 g, then manually separated based on the observed fraction of physical quality. Each fraction that has been separated is weighed, and the percentage is calculated relative to the sample's initial weight.

Damaged kernels are corn kernels that have suffered visible physical damage or changes in conditions due to handling, drying, storage, or other factors that render them no longer in an intact, normal condition. The fraction of damaged kernels is manually separated from the 100 g sample, weighed, and expressed as a percentage of the sample's total weight.

Broken kernels are incomplete corn kernels, including fragments smaller than normal kernels. The fraction of broken kernels is manually separated from the sample, weighed, and expressed as a percentage of the sample's initial weight. This parameter is used because kernel fragments can increase the surface area susceptible to further damage and mold colonization.

Moldy kernels are corn kernels that show visible mold growth on their surfaces. Identification is carried out through visual observation and, if necessary, with a magnifying glass to confirm the presence of mold growth symptoms. The moldy kernels were separated from a 100 g sample, weighed, and expressed as a percentage of the total sample weight.

Foreign materials include all components other than corn kernels contained in the sample, such as soil, small stones, plant residues, bark, dust, or other organic and inorganic materials. All foreign materials are manually separated, weighed, and expressed as a percentage of the sample's total weight. The value of each physical quality parameter is then used to classify corn quality before aflatoxin risk prediction modeling is carried out.

2.3.3. Aflatoxin Quantitation: Aflatoxin levels were evaluated using Near Infrared Spectroscopy (NIRS) with a FOSS NIR System 5000 spectrometer. Prior to analysis, maize samples were finely ground to pass through a 1 mm sieve and thoroughly homogenized. Approximately 50 g of each sample was transferred into a clean quartz cup with a flat and air-free surface. Instrument calibration was performed using standard reference materials to ensure measurement reliability prior to sample scanning. Spectral data were collected over the wavelength range 1,100–2,500 nm at 2 nm resolution. The reflectance spectra obtained were automatically processed by the instrument software and compared with the calibration library for poultry feed ingredients. Each sample was analyzed in duplicate.

The NIRS approach was used as a rapid screening method to estimate aflatoxin contamination in maize samples. The calibration model embedded in the software had previously been established by the manufacturer using reference analytical techniques, including HPLC and/or ELISA. Nevertheless, detailed calibration information such as the coefficient of determination (R^2), root mean square error of prediction (RMSEP), bias, validation dataset, limit of detection (LOD), and limit of quantification (LOQ) was not available because the calibration database was proprietary. Consequently, the aflatoxin values generated in this study should be regarded as estimated screening results rather than absolute quantitative measurements.

2.4. Classification of Aflatoxin Quality and Risk Parameters

The classification of corn physical quality parameters was established in accordance with the Indonesian National Standard SNI 8926:2020 for Corn as Animal Feed. The classified parameters included moisture content (MC), damaged kernels (DK), broken kernels (BK), moldy kernels (MK), and foreign material (FM). Each parameter is grouped into a quality class based on the SNI threshold limit, and then ordinally coded for ordinal logistic regression analysis. In addition to the Premium, Medium I, and Medium II quality classes, this study added a Non-Category (NC) class to accommodate samples with parameter values exceeding the maximum for Medium II. The NC class represents corn that falls outside the SNI quality standard and is treated operationally as the class with the lowest physical quality. This class was treated as the lowest physical quality category in the analysis. Accordingly, all physical quality parameters were coded on an ordinal scale from 1 to 4, with higher scores indicating poorer physical quality. This coding scheme enabled the use of physical quality parameters as ordinal predictors in the ordinal logistic regression model.

The level of aflatoxin risk is classified based on the characteristics of field observation results. Since aflatoxin measurements were performed semi-quantitatively using UV-based portable devices, the values obtained were not uniformly distributed but clustered around several estimated values: 20, 25, 30, and 35 ppb. Therefore, aflatoxin is treated as an ordinal response variable rather than a continuous variable. In this study, the risk of aflatoxin was

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grouped into three ordinal classes. The value of 20 ppb is categorized as risk class 1, the value of 25 ppb as risk class 2, while the value of 30 and 35 ppb is combined as risk class 3. The aggregation of values at 30 and 35 ppb was performed because the number of observations at these levels was relatively limited, so grouping was necessary to reduce the problem of classes that were too small and to improve the stability of the model (Table 1).

Table 1: Ordinal classification of aflatoxin risk based on semi-quantitative estimation results

Estimated Value of Aflatoxin (ppb)	Risk Class	Ordinal Code
20	Low risk	1
25	Medium risk	2
30–35	High risk	3

2.5. Development of Prediction Models

Ordinal logistic regression is appropriately used to analyze sequential categorical response variables because this model preserves sequence information between categories without assuming that the distances between categories are numerically equal (Agresti, 2010; Harrell, 2015). The initial model was built by including all physical quality parameters of corn as predictors: MC, DK, BK, MK and FM (Table 2). All predictors were used as ordinal quality classes based on the classification results from the previous stage. The general form of the cumulative logit model used is as follows.

$$\text{logit}[P(Y \leq j)] = \log\left(\frac{P(Y \leq j)}{1 - P(Y \leq j)}\right) = \theta_j - (\beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n)$$

Where:

$P(Y \leq j)$ = cumulative probability in the category of j

θ_j = threshold value for the category j

β_n = regression coefficient for predictor n

X_n = predictor variable, namely the physical quality class of corn.

Because the response variable had three ordinal risk classes, the model estimated two thresholds to derive cumulative and class-specific probabilities, with the highest probability assigned as the predicted class. Predictors were selected sequentially using the Wald test, and non-significant predictors (P>0.05) were removed to obtain a parsimonious model. Model adequacy and predictive performance were assessed using model fit statistics, pseudo R^2, and validation procedures (Fig. 1).

2.6. Model Evaluation and Validation

Model evaluation was conducted to assess the statistical adequacy of the ordinal logistic regression model before it was used for prediction. The model was evaluated using three main components: model-fitting information, goodness of fit, and pseudo-R-squared value.

Table 2: Criteria for evaluation and validation of aflatoxin risk prediction models

Evaluation Components	Indicator Statistics	Admission Criteria/ Threshold
Initial model qualifications	*Model fitting information* / Likelihood ratio test	P<0.05
Model compatibility with data	Pearson Goodness-of-Fit	P>0.05
	Deviance Goodness-of-Fit	P>0.05
Predictor contribution	Wald Test	P>0.05
Predictor selection	Wald Test	P>0.05
Explanatory capabilities of the model	Cox and Snell, Nagelkerke, McFadden pseudo (R2)	The higher the better
Classification validation	Classification accuracy	≥70%
Predictive–actual agreement	Cohen's Kappa	k≥0.40
Kappa Significance	Approximate significance	P>0.05
Performance stability	Progressive validation	≥70%; κ≥0.40

Model-fitting information compared the predictor model with the intercept-only model using the −2 Log Likelihood and the likelihood ratio test, where a significant result indicates improved model fit. Model adequacy was further assessed using goodness-of-fit statistics and pseudo-R^2 measures provided in the SPSS ordinal regression output, including Cox and Snell, Nagelkerke, and McFadden (Agresti, 2010; Harrell, 2015).

Goodness-of-fit is used to assess whether the constructed model conforms to the observation data pattern, and to detect model mismatches, including possible imprecise model specifications or ordinal response structures that are not well captured by the model (Fagerland & Hosmer, 2017). Pseudo-R^2 was used as a supporting measure of the model's relative explanatory ability for the categorical response, but it was not interpreted in the same way as R^2 in linear regression.

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Model validation was performed using the confusion matrix, classification accuracy, and Cohen's Kappa, following the evaluation criteria presented in Table 2. Classification accuracy measured the proportion of correctly predicted samples, while Cohen's Kappa assessed agreement between actual and predicted classes after accounting for chance agreement (Cohen, 1960; Landis & Koch, 1977; Tharwat, 2021).

In addition to initial validation, progressive validation was conducted with increasing sample sizes (N = 10, 20, 30, ..., 200) to assess the stability of model performance. This approach is conceptually aligned with learning-curve-based evaluation, in which classification performance is examined as a function of sample size to assess stability and generalization tendencies (Figueroa et al., 2012; Riley et al., 2021; Zaloumis et al., 2025). Classification accuracy and Cohen's Kappa were used as the main indicators. The model was considered stable when validation performance did not show a systematic decline as sample size increased and remained within the predefined evaluation criteria. Cohen's Kappa was interpreted according to Landis & Koch (1977), with values of 0.41–0.60 indicating moderate agreement, 0.61–0.80 indicating substantial agreement, and 0.81–1.00 indicating almost perfect agreement.

3. RESULTS

3.1. Statistical Description

Descriptive statistics of the physical quality parameters of corn and the estimation of aflatoxin contamination are presented in Table 3 and Fig. 2. In general, the observation results show considerable variation in physical quality among corn samples received as feed raw materials.

Table 3: Descriptive statistics of corn physical quality parameters and aflatoxin concentration

Parameter	Minimum	Maximum	Mean ± SD
Moisture Content	12.70	35.70	24.48 ± 5.21
Damaged Kernels	0.27	4.80	1.44 ± 0.86
Broken Kernels	0.27	2.59	1.09 ± 0.36
Moldy Kernels	0.62	5.03	2.78 ± 1.36
Foreign Material	0.27	1.53	0.95 ± 0.23
Aflatoxin (ppb)	20.00	35.00	25.48 ± 4.24

The moisture content of corn ranges from 12.70–35.70%, with an average of 24.48 ± 5.21%. The average value is far above the quality limit of feed corn moisture content recommended in SNI 8926:2020. The parameters of moldy kernels also showed relatively high variation, with values of 0.62–5.03% and an average of 2.78 ± 1.36%. Meanwhile, damaged kernels, broken kernels, and foreign materials showed lower averages than water content and moldy kernels. Damaged kernels have an average of 1.44 ± 0.86%, broken kernels 1.09 ± 0.36%, and foreign materials 0.95 ± 0.23%. Although the value is relatively low, the three parameters remain relevant to raw material quality. Aflatoxin concentration ranged from 20–35 ppb, with an average of 25.48 ± 4.24 ppb.

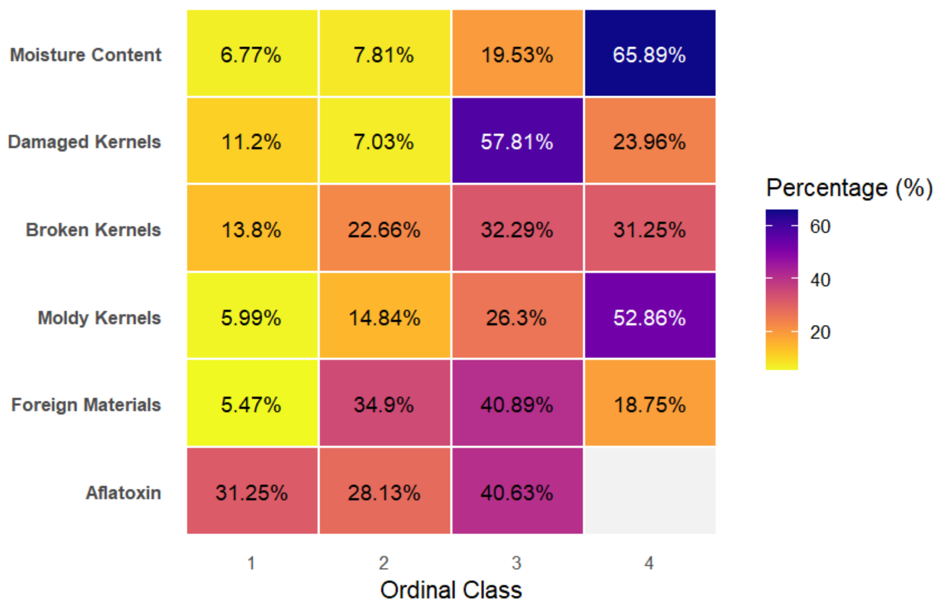

Fig. 2: Heat map of corn physical quality parameters and aflatoxin risk by ordinal class.

The ordinal class distribution pattern shows that moisture content and moldy kernels had the largest sample concentrations in class 4, at 65.89 and 52.86%, respectively, indicating a dominance of poor-quality samples. Damaged kernels were also concentrated in class 3 at 57.81%, while broken kernels were relatively evenly distributed across classes 3 and 4. Meanwhile, foreign objects are more in classes 2 and 3. For aflatoxin, the highest proportion

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was in risk class 3 at 40.63%. The heatmap indicated that most samples were in post-harvest quality conditions and were of low quality, characterized by high moisture content, physical damage, and the presence of moldy kernels, which were descriptively consistent with an increased risk of aflatoxin contamination.

3.2. Key Physical Quality Indicators

An initial ordinal logistic regression model was developed. At this initial stage, all predictors (MC, DK, BK, MK, and FM) were included simultaneously to evaluate the contribution of each physical quality parameter to the chance of an increase in aflatoxin (AFL) risk class.

The initial estimation model result (Table 4) shows that not all physical quality parameters contribute significantly to aflatoxin risk prediction. Based on the Wald test, three variables have a significant effect: moisture content (B = -2.407; p = 0.007), broken kernels (B = 0.619; P = 0.006), and moldy kernels (B = 2.315; P<0.001).

Among the significant variables, moldy kernels showed the strongest association, with an Exp(B) of 10.125. Broken kernels showed a positive and significant association with aflatoxin risk class, with an Exp(B) value of 1.857. In contrast, moisture content showed a significant negative coefficient, with an Exp(B) value of 0.090, indicating a negative association with higher aflatoxin risk class in the multivariate model.

Meanwhile, damaged kernels and foreign materials did not show significant effects on aflatoxin risk, with p-values of 0.267 and 0.713, respectively. Therefore, damaged kernels and foreign materials were excluded from the subsequent model, while moisture content, broken kernels, and moldy kernels were retained as statistically contributing predictors.

Table 4: The results of the estimation of the initial model of ordinal logistic regression with all physical quality parameters of corn

Variable	Coefficient (B)	SE	Wald	Sig.	Exp. (B)
MC	-2.407	0.889	7.329	0.007[i]	0.090
DK	0.599	0.540	1.230	0.267[e]	1.820
BK	0.619	0.226	7.490	0.006[i]	1.857
MK	2.315	0.498	21.642	0.000[i]	10.125
FM	0.086	0.233	0.135	0.713[e]	1.090

Note: [i]= included in the final model; [e] = excluded from the final model. B = logit coefficient; Exp(B) = odds ratio.

3.3. Aflatoxin Risk Prediction Model

***3.3.1. Prediction Model*:** The feasibility of the final model was evaluated using the fitting information, goodness-of-fit, and pseudo R-square model (Table 5). The model fit test yielded a Chi-square value of 236.509 with P<0.001, indicating that the final model with predictors provided a better fit than the base model containing only intercepts.

Table 5: End-to-end model feasibility indicators of ordinal logistic regression

Test Components	Indicators	Value	Sig.
Model Fitting	-2 Log Likelihood (Final)	73.505	-
	Chi-Square	236.509	<0.001
Goodness-of-Fit	Pearson Chi-Square	18.900	0.651
	Deviance Chi-Square	19.640	0.606
Pseudo R^2	Cox and Snell	0.460	-
	Nagelkerke	0.519	-
	McFadden	0.284	-

Note: Sig. = significance value; pseudo R^2 is used as a measure of relative explainability in models with ordinal categorical responses and is not interpreted to be the same as R^2 in linear regression.

Pearson's chi-square and Deviance Chi-square were insignificant with P = 0.651 and P = 0.606, respectively. A p-value greater than 0.05 indicates that there is no significant difference between the observed data and the constructed model. In other words, the final model can be considered to have an adequate fit for the data structure being analyzed. The pseudo-R-squared value supports the model's feasibility. The Cox and Snell value of 0.460, Nagelkerke of 0.519, and McFadden of 0.284 indicate that the model has a fairly strong relative explanatory power in distinguishing aflatoxin risk classes. In particular, the Nagelkerke value of 0.519 indicates that the model makes a good contribution to explaining ordinal categorical data, although it is not directly comparable to R^2 in linear regression.

3.3.2. Risk Prediction Framework: The final model of ordinal logistic regression in this study was formulated using the cumulative logit model approach. On ordinal responses with categories, the cumulative logit model forms ***c****c – 1* Logit functions that represent the cumulative chance of a response are at or below a specific category. (Agresti, 2010; Harrell, 2015) explains that the cumulative logit model is used to model $P(Y \leq j)$, where j = 1, 2, ..., $c - 1$, so this model is appropriate for responses that have a category sequence. In this study, the AFL response variable comprises three risk classes, so the final model yields two cumulative logit functions: P(AFL ≤ 1) and P(AFL ≤ *2*). In general, the cumulative logit model obtained can be written as follows:

$$\text{logit}[P(AFL \leq j)] = \theta_j - (\beta_1 MC + \beta_2 BK + \beta_3 MK)$$

With $j = 1, 2$

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In the equation, *P(AFL≤ j)* shows the cumulative probability that the sample is in the aflatoxin risk class up to the jth category, which is the threshold value or category threshold, while θ_j *β1*, *β2*, and *β3* are the regression coefficients for each predictor. The variables MC, BK, and MK represent the ordinal classes of moisture content, broken kernels, and moldy kernels. Since there are three classes of aflatoxin risks, the model produces the following two cumulative logit equations:

$$\text{logit}[P(AFL \leq 1)] = \theta_1 - (\beta_1 MC + \beta_2 BK + \beta_3 MK)$$

$$\text{logit}[P(AFL \leq 2)] = \theta_2 - (\beta_1 MC + \beta_2 BK + \beta_3 MK)$$

The first equation was used to estimate the cumulative probability that a sample is in a low-risk class, while the second equation was used to estimate the cumulative probability that a sample is in a low- to moderate-risk class. Furthermore, the probability for each risk class is derived from the cumulative probability. The predictor coefficient indicates the change in the cumulative probability of aflatoxin risk classes per 1-unit increase in physical quality parameters, holding other predictors constant. Therefore, the model not only produces the final classification but also provides a probabilistic estimate of the likelihood that a corn sample belongs to a given risk class, based on the combination of MC, BK, and MK values.

***3.3.3. Estimation of Risk Class Probability*:** Once the cumulative logit value is obtained from the final model equation, the probability of each aflatoxin risk class can be calculated through a logistic transformation. Since the response variable consists of three ordinal classes, the model produces two cumulative probabilities: the probability that the sample is in a risk class of 1 or lower and the probability that the sample is in a risk class of 2 or lower. Mathematically, the cumulative probability is calculated as follows:

$$P(AFL \leq j) = \frac{\exp(L_j)}{1 + \exp(L_j)}$$

with:

$$L_j = \text{logit}[P(AFL \leq j)]$$

In the equation, it is the cumulative logit value at the boundary of the *j*-th class, while it shows the cumulative probability up to the risk class of-. Since there are three classes of risk, the cumulative probability calculated is: $L_j\, j P(AFL \leq j) j$

$$P(AFL \leq 1)$$

and

$$P(AFL \leq 2)$$

Furthermore, the probability of each risk class is derived from the cumulative probability. The probabilities of risk classes 1, 2, and 3 are calculated as follows:

$$P(AFL = 1) = P(AFL \leq 1)$$

$$P(AFL = 2) = P(AFL \leq 2) - P(AFL \leq 1)$$

$$P(AFL = 3) = 1 - P(AFL \leq 2)$$

Each corn sample is classified into a single risk class and assigned a probability for that class. The class with the highest probability is then determined as the predicted outcome risk class. For example, if a sample has probabilities of 0.20 for low risk, 0.35 for medium risk, and 0.45 for high risk, then the sample is classified as high risk because it has the highest probability.

3.4. Aflatoxin Prediction Model Validation

Model validation shows that 77 of 105 validation samples were correctly classified, yielding an overall accuracy of 73.33%. Based on the confusion matrix (Table 6), the model achieves high accuracy in predicting risk classes 1 and 3, with accuracies of 89.47 and 80.00%, respectively. Meanwhile, risk class 2 had a lower accuracy of 46.88%. This suggests that the medium-risk class is the most difficult category to predict because it represents transitional conditions between low and high risk.

Table 6: Confusion matrix validation results of the aflatoxin risk prediction model

Actual Class	Predictions			Total	Class Accuracy (%)
	Class 1	Class 2	Class 3		
Class 1	34	3	1	38	89.47
Class 2	7	15	10	32	46.88
Class 3	2	5	28	35	80.00
Total	43	23	39	105	73.33

Table 7: Summary of ordinal logistic regression model validation based on classification accuracy and Cohen's Kappa

Indicators	Value
Number of samples (N) Validation test	105
Accurate prediction	77
Classification accuracy	73.33%
Most accurate class	89.47%
Unpredictable classes	46.88%
Cohen's Kappa	0.597
Std. Error	0.086
Value z (Approx. T)	7.786
Significance (p-value)	<0.001

Cohen's Kappa calculation results show a Kappa value of 0.597, which falls in the moderate agreement category (Table 7). This value indicates that the match between the prediction results and the actual data is not due to chance but reflects a fairly good level of predictive reliability.

The progress validation results indicate that classification accuracy ranged from 74.50% to 83.20%. At small sample sizes, accuracy tends to fluctuate: 79.50% at N=10, 82.00% at N=20, then 74.50% at N=40. These fluctuations suggest that evaluations with small sample sizes can yield less stable performance estimates. As the number of validation samples increases, the model's accuracy tends to be more stable. At N ≥ 120, the accuracy value was in the range of 77.80–83.20%, and at N ≥ 150 the accuracy was relatively consistent above 82%. Cohen's Kappa scores show a clearer pattern of improvement than accuracy. At N = 10, the Kappa value is 0.286, indicating fair agreement. The value increased to 0.448 at N = 30 and was in the moderate agreement range for most subsequent sample sizes. At N ≥ 130, the Kappa value was relatively stable, ranging from 0.549 to 0.586, suggesting that the fit between the actual and predicted classes became more consistent as the validation sample size increased (Fig. 3).

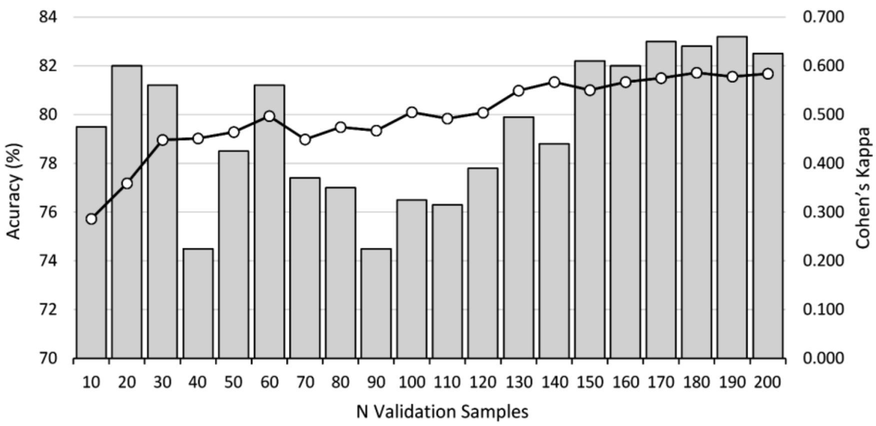

Fig. 3: Progressive validation of the ordinal logistic regression model across increasing validation sample sizes (N = 10–200), showing classification accuracy and Cohen's Kappa.

4. DISCUSSION

This study found that the physical quality of corn as a feed raw material was relatively heterogeneous, particularly in moisture content and the presence of moldy kernels. The wide range and high average values of moisture content indicate that some samples had not yet reached stable conditions for storage, making them more susceptible to heating, microbial activity, and degradation during handling (Domenico et al., 2015; Suleiman et al., 2018; Muga et al., 2019; Kassa et al., 2025). The relatively prominent moldy kernels reinforce indications that microbiological deterioration has occurred in some raw materials. Damaged kernels, broken kernels, and foreign objects have lower average values, but still provide information on the quality of post-harvest handling, sorting processes, transportation, and cleanliness of raw materials in the corn supply chain. The concentration of aflatoxin in the range of 20–35 ppb, with an average of 25.48 ± 4.24 ppb, indicates that contamination risk has been detected since the raw material acceptance stage. This pattern supports the view that aflatoxin control in feed corn needs to start from strengthening drying, sorting, segregation, and storage management from the supply chain, as emphasized in a postharvest mitigation study on corn (Odjo et al., 2022; Matusse et al., 2025). In the context of the feed industry, physical quality parameters can serve as an initial signal to sort raw materials that need to be re-dried, sorted, further tested, or rejected before entering the plant's storage system.

The final model shows that aflatoxin risk was explained more strongly by the combination of moisture content, broken kernels, and moldy kernels than by all other observed physical quality parameters. The inclusion of these three variables in the final model can be interpreted as reflecting the risk of aflatoxin formation through two main pathways: microenvironmental conditions that support mold development and the physical vulnerability of the kernels due to structural damage. In the model, the moisture content represents the micro-conditions related to mold growth opportunities and toxin production. The relationship needs to be understood together with the factors of time, water activity, and temperature, as shown in the time–water activity–temperature aflatoxin B1 production model (Marín et al., 2024). Broken kernels provide information about the loss of the physical integrity of the kernel, so it is more appropriately positioned as an indicator of material susceptibility (GuiXiang et al., 2023), rather than the sole cause of contamination. Meanwhile, moldy kernels have a more direct biological affinity with mold activity, especially since Aspergillus flavus infection of corn kernels involves changes to the surface and structure of the kernels (Lu et al., 2024). This pattern is in line with the latest mycotoxin modeling approach that places a variety of risk indicators, ranging from pre-harvest and post-harvest practices, environmental conditions, to material characteristics, as complementary sources of information in explaining contamination variations in corn (Stafstrom et al., 2025).

Moldy kernels have the strongest biological association with aflatoxin risk in the final model (B = 2,315; exp(B) = 10.125; Table 4). This variable indicates that some kernels had reached a condition conducive to fungal activity. In the model results, an increase in the moldy kernel class was associated with a higher probability that samples would be classified into a higher aflatoxin risk category. This statistical pattern is consistent with the biological logic of corn contamination, although visible mold does not necessarily indicate aflatoxin production in every case. Aflatoxin-producing fungi, particularly those from the *Aspergillus* genus, can grow on food and feed materials under favorable storage conditions, and the presence of fungi in kernels is also associated with quality deterioration and potential mycotoxin formation (Benkerroum, 2020; Martín et al., 2022). In stored corn, factors such as Aspergillus and moisture content, along with kernel characteristics, have also been used to explain the likelihood of aflatoxin emergence (Domenico et al., 2015). Recent findings on the aflatoxigenic interactions of *Aspergillus flavus* with corn kernels reinforce this reading, as the infection process involves dynamics at the surface and within the kernels' structure (Lu et al., 2024).

Broken kernels in the final model showed a positive association with aflatoxin risk class, as indicated by the positive coefficient and Exp(B) of 1.857 in the ordinal logistic regression model. These findings place kernel breakage as an indicator of the physical vulnerability of raw materials, especially since *kernel breakage* has been reported as a form of corn quality degradation related to mechanical stress, moisture content, and post-harvest handling processes (GuiXiang et al., 2023; Shahbazi & Shahbazi, 2018). When the kernel structure is damaged, the protective physical barrier is reduced, exposing the inner surface and increasing the susceptibility of corn kernels to deterioration during storage (Shahbazi & Shahbazi, 2018). Especially aflatoxin contamination, this condition needs to be read along with other factors such as moisture content, storage conditions, and the presence of mold, because the chance of aflatoxin appearing in stored corn is influenced by a combination of physicochemical and microbiological parameters (Domenico et al., 2015; Lu et al., 2024).

Moisture content is a predictor that requires careful interpretation in this model. Statistically, moisture content was significantly associated with aflatoxin risk class, but the coefficient was negative (B = -2.407; Exp(B) = 0.090; Table 4). This negative coefficient likely reflects that the moisture content measured at the receiving point is not a single marker of the contamination risk history; it does not represent the corn's moisture history during the post-harvest process. In other words, the aflatoxin detected at the time of receipt may be the result of prior conditions (harvesting, drying, storage, and transportation), rather than solely due to the actual moisture content at the time the sample was analyzed. In addition, the model included moldy kernels; some of the biological information about the impact of moisture on mold development may have been captured by this variable. Therefore, the negative coefficient for water content is better understood as a statistical pattern after controlling for other quality indicators, rather than as a direct biological relationship between water content and aflatoxin.

The exclusion of damaged kernels and foreign materials in the final model should be understood proportionally. These results do not imply that the two parameters are irrelevant to the quality assessment of corn. Damaged kernels and foreign materials remain relevant as indicators of physical quality, raw material cleanliness, and post-harvest handling quality. However, its role as a predictor of aflatoxin risk in this model appears weaker, indirect, or captured by other parameters that are closer to the biological processes underlying risk formation, especially broken and moldy kernels. The final model, therefore, should be understood as a predictive simplification: it retains the indicators that most strongly explain variation in aflatoxin risk within the dataset analyzed, while other physical quality parameters remain relevant to broader corn quality control.

Statistically, the final model demonstrates adequate feasibility as an early predictor model of aflatoxin risk. The results of the fitted model showed improvement over the baseline model without predictors, while the insignificant goodness-of-fit value indicated that the model conformed to the observed data pattern. The pseudo-R-square values, especially Nagelkerke, indicate that the combination of moisture content, broken kernels, and moldy kernels makes a fairly strong explanatory contribution to variation in aflatoxin risk class (Table 5). However, pseudo-R-squared in categorical models should be interpreted as a relative measure of model improvement, not as a coefficient of determination in the same sense as in linear regression. The probabilistic approach in the model is important because the risk of aflatoxin may fall within transitional conditions, especially when the physical quality parameters lie between clearly acceptable and clearly poor-quality limits. The validation results (Table 6; Fig. 3) support the model's use as an initial screening tool. The classification accuracy exceeded the evaluation limit, Cohen's Kappa indicated agreement beyond chance, and progressive validation showed relatively stable performance as the validation sample size increased. However, predictive ability varies across classes; low- and high-risk classes are easier to recognize, while medium-risk classes tend to be transition zones that are more difficult to classify.

This research occupies a specific position within the development of aflatoxin prediction models in corn. Previous studies have utilized meteorological data, vegetation indices, geospatial information, Bayesian networks, mechanistic models, and machine learning to estimate aflatoxin risk at the level of corn production areas or planting systems (Castano-Duque et al., 2022; Liu et al., 2022; Stafstrom et al., 2025). These approaches are useful for early warning, particularly when the risk of contamination is affected by climatic conditions, production sites, and environmental dynamics. The feed industry, however, faces a more operational question: how to assess raw material risk rapidly when corn is received at the mill. The model developed in this study addresses this decision point by using physical quality parameters routinely available during raw material inspection: moisture content, broken kernels, and moldy kernels. The model is not intended to replace quantitative aflatoxin testing but to serve as an early screening tool to identify corn lots that may require redrying, sorting, segregation, further testing, or rejection before entering the industrial storage system.

5. CONCLUSION

This study shows that the physical quality parameters of corn can be used as the basis for the development of an aflatoxin contamination risk prediction model based on ordinal logistic regression. The final model retains three main predictors: moisture content (MC), broken kernels (BK), and moldy kernels (MK), while damaged kernels and foreign materials do not contribute statistically significantly. The evaluation of the model shows that it is statistically sound, as evidenced by a significant fit and a relatively strong pseudo-R-squared value. Model validation on 105 samples yielded a classification accuracy of 73.33%, with the highest accuracy in the low-risk class, and the medium-risk class being the most difficult to predict. Cohen's Kappa score indicates a moderate degree of agreement between the actual class and the predicted class.

Progressive validation from N = 10 to N = 200 shows that model performance is relatively stable, with accuracy in the range of 74.50–83.20%, and Cohen's Kappa tends to increase, reaching a more stable moderate agreement category at larger sample sizes. Thus, the ordinal logistic regression model developed can be used as an early warning tool to identify the risk of aflatoxin contamination in corn based on physical quality parameters. This model is not intended to replace confirmatory laboratory testing, but it can support rapid decision-making in the quality control of feed raw materials.

Declarations

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