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CO-OCCURRENCE OF AFLATOXINS, OCHRATOXIN A AND ZEARELENONE IN FEED COMPONENTS, DETERMINED BY LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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Introduction. Mycotoxins are secondary metabolites secreted by moulds, mostly *Aspergillus*, *Penicillium* and *Fusarium*. Aflatoxins, ochratoxin A and zearalenone are the most prevalent and predominant. They can contaminate agricultural commodities during and after harvest and storage in various environmental conditions. Mycotoxins deserve special attention because more than 25% of the world's crops are infected each year. IARC made classification and most of them are carcinogen to humans. Content of mycotoxins in feedstuffs is regulated by legislation worldwide with MRL in the range of 50-3000 µg/kg.

Materials and Methods. Seventy (70) samples of feed components (30 corn, 19 wheat, 9 barley, 10 soybean pellets and 2 sunflower pellets) were analyzed for the presence of aflatoxins, OTA and ZEA. For the experiment purposes, mycotoxin - free samples were spiked with known amount of proposed mycotoxins at three concentration levels, depending on the type of the mycotoxin. The extraction of samples was done according to several modified AOAC and ISO methods. For clean-up procedure immunoaffinity columns were applied. Perkin Elmer HPLC-FD system was employed.

Results and Discussion. Conventional validation approach includes linearity, recovery, repeatability, within-laboratory reproducibility, limit of detection and limit of quantification according to Regulation 401/2006/EC and Decision 2002/657/EC. Parameters provide satisfactory values for all performing criteria for all three methods. The methods were linear and a good coefficients of correlation (R^2) were found (0,9993-0,9999). LOD (0,003–1,34 µg/kg) and LOQ (0,009–4,06 µg/kg) were acceptable and in accordance with the literature. Recovery was performed according to the method of standard addition and the results are in the range of 88,22-115,28%. RSD values were used for estimation of repeatability and RSDr were in the range of 0,17-11,35%. Within-laboratory reproducibility show good correlation between two days. RSDR results for aflatoxins were 6,60-4,93%; for OTA 2,37-1,16% and for ZEA 3,69-1,26%. Total of 70 samples of feed components were analyzed for the presence of proposed mycotoxins. Among them, 59 samples were below LOD. Three (3) corn samples were positive on aflatoxins in accordance with legislation (MRL is 20 µg/kg) in range of 23,55-52,7 µg/kg.

Conclusions. As we can see from the results, most of the samples (84,2%) are with aflatoxins, OTA and ZEA concentration below LOD and only 3 corn samples are positive for the presence of aflatoxins. None of the samples were positive for OTA and ZEA. Although the number of positive samples is low, attention should be directed on strategies for providing feed safety in the continuous food chain.

Keywords: aflatoxins, ochratoxin A, zearalenone, feed components, validation, HPLC-FD



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ВЗАИМОДЕЙСТВИЕ АФЛАТОКСИНОВ, ОСХРАТОКСИНА И ZEAREALENONE В КОМПОНЕНТАХ КОРМА, ОПРЕДЕЛЕННЫХ ЖИДКОСТНОЙ ХРОМАТОГРАФИЕЙ С ОБНАРУЖЕНИЕМ ФЛЮОРЕСЦЕНЦИИ

Введение. Микотоксины - вторичные метаболиты, спрятавшие формами, главным образом *Aspergillus*, *Penicillium* и *Fusarium*. Афлатоксины, ochratoxinA и zearalenone являются самыми распространенными и преобладающие. Они могут загрязнить сельскохозяйственную продукцию в течение и после урожая и хранения в различных условиях окружающей среды. Мycotoxins заслуживают особого внимания, потому что больше чем 25% зерновых культур в мире заражаются каждый год. IARC сделал классификацию, и большинство из них - канцерогенное вещество людям. Содержание микотоксинов в кормах отрегулировано законодательством во всем мире с MRL в диапазоне 50-3000 мкг/кг.

Материалы и методы. Семьдесят (70) образцов компонентов подачи (30 зерен, 19 пшеницы, 9 ячменя, 10 шариков сои и 2 шарика подсолнечника) были проанализированы для присутствия афлатоксинов, ОТЫ и ZEA. В целях эксперимента мycotoxin - бесплатные образцы были пронзены с известной суммой предложенного микотоксина на трех уровнях концентрации, в зависимости от типа мycotoxin. Извлечение образцов было сделано согласно нескольким, изменил методы ISO и AOAC. Для процедуры по очистке immunoaffinity колонки были применены. Перкин Элмер система HPLC-FD.

Результаты и Обсуждение. Обычный подход проверки включает линейность, восстановление, воспроизводимость в пределах лаборатории, предел обнаружения и предел определения количества согласно Постановлению 401/2006/ЕС и Решению 2002/657/ЕС. Параметры обеспечивают удовлетворительные ценности для критериев выполнения всех трех методов. Методы линейны и которой коэффициенты корреляции (R^2) были найдены (0,9993-0,9999). ЛОД (0,003-1,34 $\mu\text{g/kg}$) и LOQ (0,009-4,06 $\mu\text{g/kg}$) приемлем в соответствии с литературой. Восстановление было выполнено согласно методу стандартного дополнения, результаты находятся в диапазоне 88,22-115,28%. Ценности RSD использовались для оценки воспроизводимости, и RSDr были в диапазоне 0,17-11,35%. Воспроизводимость в пределах лаборатории хорошая корреляция между двумя днями. Результатами RSDr для афлатоксинов составляли 6,60-4,93%; для ОТЫ 2,37-1,16% и для 3,69-1,26% ZEA. Общее количество 70 образцов компонентов подачи было проанализировано для присутствия предложенного микотоксина. Среди них 59 образцов были ниже ЛОДА. Три (3) образца зерна были положительными относительно афлатоксинов в соответствии с законодательством (MRL - 20 $\mu\text{g/kg}$) в диапазоне 23,55-52,7 $\mu\text{g/kg}$.

Заключение. Как мы видим большинство образцов (84,2%) с афлатоксинами, ОТОЙ и концентрацией ZEA ниже ЛОДА, и только 3 образца зерна положительные. Ни один из образцов не был положительным для ОТЫ и ZEA. Хотя число положительных образцов низкое, внимание должно быть направлено на стратегиях обеспечения безопасности подачи в непрерывной пищевой цепи.

Ключевые слова: афлатоксины, ochratoxin A, zearalenone, кормя компоненты, проверку, HPLC-FD

Ключевые слова: *Listeria monocytogenes*, вирулентность генов, бойня.