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Research Article

Evaluation of Aflatoxin B1 Binding Capacity with Mix Toxin Binder using Central Composite Design

Nihayet Fadime Yalçın[a,1], Cafer Cantekin[a,2], Halis Uğuz[b,3], Mustafa Selcuk Alatas[b,4], Syed Tufail Hussain Sherazi[c,5], Mustafa Topkafa[d,6*]

[a] *Toxicology Laboratory, Konya Veterinary Control Institute, Ministry of Food, Agriculture and Livestock, Konya 42080, Turkiye, ror.org/04cdzx077*
[b] *Veterinary Faculty, Selcuk University, Konya 42075, Turkiye, ror.org/045hgzm75*
[c] *National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro-76080, Pakistan, ror.org/01d692d57*
[d] *Konya Technical University, Vocational School of Technical Sciences, Department of Chemistry and Chemical Processing Technologies, 42075 Campus, Konya/Turkiye, ror.org/02s82rs08*

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ABSTRACT

Aflatoxin B1 (afl B1) binding capacity of a mixed toxin binder used in poultry nutrition were determined using the central composite design technique. Experimental conditions such as pH, temperature and incubation time for the determination of the binding capacity of a mix binder were optimized for Central Composite Design. The impact of these three independent variables on the % binding of aflatoxin B1 was evaluated at different five levels (-1.68, -1, 0, 1, 1.68). The optimum experimental conditions were 5.8, 42°C, 94.11 min for pH, temperature and incubation time, respectively using quadratic model and desirability function. A significant effect of each independent variable was observed on the % binding efficiency of aflatoxin B1. In optimum experimental conditions, aflatoxin B1 binding capacity with mix toxin binder was found 97%. The results of the present study indicated that the mix binder is very suitable for binding of aflatoxin B1 and the central composite design can be used effectively in determining the optimized parameters for improving toxin binding capacity of aflatoxin B1.

Araştırma Makalesi

Aflatoksin B1 Bağlama Kapasitesinin Karışık Toksin Bağlayıcı ile Merkezi Kompozit Tasarım Kullanılarak Değerlendirilmesi

MAKALE BİLGİSİ

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ÖZ

Kanatlı beslenmesinde kullanılan karışık bir toksin bağlayıcı olan aflatoksin B1`in bağlama kapasitesi, merkezi kompozit tasarım tekniği kullanılarak belirlenmiştir. Karışım bağlayıcının bağlama kapasitesinin belirlenmesi için pH, sıcaklık ve inkübasyon süresi gibi deneysel koşulların optimizasyonu için merkezi kompozit tasarım kullanılmıştır. Bu üç bağımsız değişkenin aflatoksin B1'in bağlanma yüzdesi üzerindeki etkisi beş seviyede (-1.68, -1, 0, 1, 1.68) uygulanmıştır. Bu değişkenlerin 3 boyutlu grafikler dikkate alındığında aflatoksin B1 bağlanması üzerinde önemli etkiye sahip olduğu gözlenmiştir. Kuadratik model ve desirability fonksiyonu yardımıyla optimum deneysel koşullar pH 5.8, sıcaklık 42°C ve inkübasyon süresi 94.11 dakika olarak tespit edilmiştir. Her bir bağımsız değişken, aflatoksin B1'in bağlanma yüzdesi üzerinde anlamlı bir etkiye sahipti. Optimum deney koşullarında karışık toksin bağlayıcının aflatoksin B1'i %97 gibi yüksek bir oranında bağladığı belirlenmiştir. Sonuçlar, karışım bağlayıcının aflatoksin B1'in bağlanması için çok uygun olduğunu ve merkezi kompozit tasarımın toksin bağlayıcıların toksin bağlama kapasitesinin belirlenmesinde etkin bir şekilde kullanılabileceğini göstermiştir.

* Sorumlu Yazar
E-posta adresleri: nfadimeyalcin72@yahoo.com (N. F. Yalçın), cafer.cantekin@tarimorman.gov.tr (C. Cantekin), hlsgz@hotmail.com (H. Uğuz), selcukalatas@gmail.com (M. S. Alatas), tufail.sherazi@gmail.com (S. T. H. Sherazi), mtopkafa@ktun.edu.tr (M. Topkafa)
[1] ORCID: 0000-0002-5319-8650
[2] ORCID: 0000-0001-7389-8972
[3] ORCID: 0000-0002-9236-0630
[4] ORCID: 0000-0001-9920-1838
[5] ORCID: 0000-0003-0210-2759
[6] ORCID: 0000-0001-7016-798X
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Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur (M. Topkafa).

It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (M. Topkafa).

İntihal Kontrolü **Plagiarism Check**

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Çıkar Çatışması **Conflict of Interest**

Yazarlar, bu makalede bildirilen çalışmayı etkiliyor gibi görünebilecek bilinen hiçbir rakip mali çıkarları veya kişisel ilişkileri olmadığını beyan ederler.

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1. Introduction

Mycotoxins are secondary metabolites produced by the growth of various fungal species under suitable environmental conditions (humidity, temperature, etc.) during processes such as harvesting and storage conditions, climate change, inappropriate drying and packaging (Milani, 2013; Mannaa and Kim, 2017; Kovač et al., 2018). Aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, fumonisin B1 and trichothecenes (T-2 and HT-2) are the most important ones out of 400 identified mycotoxins in terms of their prevalence, economic aspects and negative effects on public health (Pitt, 2000; Eskola et al., 2020). While these mycotoxins can cause digestive disorders, oral lesions, immunological effects, hematological disorders and cancer, they exhibit many toxic effects, including teratogenicity, mutagenicity, nephrotoxicity, hepatogenicity, and genotoxicity (Frizzell et al., 2011; Bui-Klimke and Wu, 2015; Rushing and Selim, 2019). In mycotoxicosis, the severity of symptoms varies according to animal species, sex, age, exposure time, mycotoxin type, current level, and synergistic effect of these parameters by being present in more than one number (Schiefer, 1990).

Contamination of food and feed with mycotoxins is an important problem all over the world. They constitute a major risk factor for human and animal health. For this reason, various methods such as physical, chemical and biological are used in the detoxification of mycotoxins in order to minimize the negative effect (Gomaa et al., 1997; Raters and Matissek, 2008; Calado et al., 2014; Trombete et al., 2017; İPÇAK et al., 2019; Lyagin and Efremenko, 2019; Taheur et al., 2019). Binders, which are feed additives, are widely preferred by breeders and feed industry (De Mil et al., 2015). Binders are used to prevent clumping in feeds, but they also reduce toxin absorption from the digestive system by adsorbing mycotoxins. The toxin complex formed by toxin binders is then excreted in the feces. Toxin binders are basically classified as organic (yeast cell wall and glucomannan) and inorganic binders (clay minerals such as aluminosilicate, bentonite and zeolite) (Vila-Donat et al., 2018).

Feed additives can be used in animal nutrition to improve feed quality, performance and health of animals. These substances, which are scientifically proven to have no harmful effects on human and animal health and the environment, can be placed on the feed market with the permissions granted. The properties of the additives such as purity, physical properties (dusting potential, particle size, distribution, etc.), efficiency and reliability must be in the desired conditions (HUB; Commission, 1998; Additives et al., 2018). In the present work, central composite design as an experimental design was used to evaluation of % binding of aflatoxin B1 capacity for a mix toxin binder used in poultry nutrition.

2. Materials and Methods

2.1. Chemicals and standards

The all chemicals in the analyzes were chromatographic purity and were purchased from Merck and Sigma-Aldrich. The toxin binder which has 20% yeast and 80% active clinoptilolite was provide from local market in Konya, Turkey. The aflatoxin B1 stock solution, which was purchased from Sigma-Aldrich, were prepared in amber bottle to 10 ppm and stored at +4°C until test was performed. Solutions that have different pH's were prepared with HCl and NaOH.

2.2. Experimental design and desirability function (DF)

The central composite design parameters consisting of pH, time and temperature independent variables and experimental runs have 5 levels (-1.68, −1, 0, +1, +1.68) were given in Table 1. For response surface methodology (RSM), aflatoxin B1 was used as response value (RSD < 0.5%). The version 12 of the Design Expert software (Stat-Ease corporation, USA) was used for chemometric design. All samples were analyzed in triplicate (n= 3).

Table 1. CCD for the three independent variables.

Run	pH	Temperature (°C)	Incubation Time (min)	% Binding
1	6,69	41	95	87.14
2	4,5	41	69,7731	81.43
3	4,5	41	95	96.07
4	5,8	42	80	94.64
5	4,5	41	95	93.93
6	4,5	41	95	97.14
7	5,8	42	110	94.29
8	4,5	41	95	94.29
9	3,2	42	110	73.21
10	5,8	40	80	79.64
11	3,2	40	80	76.79
12	4,5	39,32	95	96.07
13	4,5	42,68	95	86.07
14	3,2	40	110	79.29
15	3,2	42	80	80.36
16	5,8	40	110	77.14
17	4,5	41	95	94.64
18	2,31	41	95	93.21
19	4,5	41	120,23	86.79
20	4,5	41	95	93.57

2.3. Sample collection and preparation

10 mg of toxin binder was weighed into 15 mL falcon tube. 50 µL stock afl B1 standard solution (final concentration of 100 ppb) and 5 mL of the pH-adjusted solution were added and vortexed. The samples were shaken in the incubator with a stirrer at the specified temperature and time. Later samples were centrifuged at 1000 rpm/min for 3 minutes. After waiting for 10 minutes, 800µL of supernatant and 800µL of methanol were added to 2mL vials, vortexed and analyzed in HPLC.

2.4. HPLC and its parameters

An Agilent 1260 series HPLC system consisted of florescence detector set at wavelengths 362 nm and 440 nm for excitation and emission, respectively, and on ACE C18 column (250× 4.6 mm, 5 µm,) was used for aflatoxin analysis. Photochemical derivatization (LC Tech -UVE) was used in

analysis. An isocratic separation of water, methanol and acetonitrile (57.2 /28.6/14.2 v/v/v/) was carried out at a flow rate of 1.0 ml min^{-1} as mobile phase

3. Results and Discussion

Working samples containing afl B1 were prepared and incubated according to central composite design. After incubation, afl B1 analysis were carried out by using HPLC. The remaining afl B1 amount were utilized as response values and 3D plots were drawn. The optimum conditions for maximum afl B1 adsorption were detected by second-order quadratic model and desirability function.

The prepared and incubated samples were subjected to HPLC analysis. The % bindings of afl B1 were calculated by using the remaining Afl B1 amounts and were taken as response values. In order to determine the most accurate design after incubations under different conditions, the coded equation was calculated using the Design Expert software program. The design program determined that the quadratic model was the most suitable among 2FI, linear, quadratic and cubic models. While determining the suitable model, high R-square (R^2), appropriate coefficient of variation (CV) and low standard deviation (S.D) values were taken into account and the following equation was obtained:

$$AflB1=+95.1382+1.89293A+0.938874B+0.11089C+4.3325AB+0.225AC-0.9375BC-2.97997A^2-2.66353B^2-5.12427C^2$$

where A, B and C are pH, temperature and incubation time, respectively (Topkafa and Ayyildiz, 2017). Statistical results obtained from an analysis of variance (ANOVA) are shown in Table 2. The adjusted R^2 and *P* for % bindings of afl B1 was calculated calculated as 0.6230, 0.1787, respectively. The S.D value of 6.70 proved the performance of the model. The CV of 7.63% indicated that the model was reasonably reproducible as the CV was not greater than 10%. [23]. In terms of % bindings of afl B1 the model, F-value of 1.84 implies the model is not significant. The Lack of Fit F-value of 46.06 implied the Lack of Fit is not significant relative to the pure error. There is only a 0.03% chance that a Lack of Fit F-value this large could occur due to noise.

Table 2. ANOVA for the model predicted.

Source	SS	DF	MS	F-value	p-Value	Comment	R^2	Std. Dev.	C.V. %
Model	740.78	9	82.31	1.84	0.1787	not significant			
Error (Residual)	448.29	10	44.83						
Lack of Fit	438.77	5	87.75	46.06	0.0003	significant	0.6230	6.70	7.63
Pure Error	9.53	5	1.91						
Total	1186.08	19							

By using regression models, the 3D surface plots were plotted for % bindings of afl B1. The 3D plots indicate the effects of pH, incubation time and temperature on % binding of afl B1. In 3D plots (Fig 1), one factor is kept constant at the center (0) while the other two factors are changed. The effect of pH (A) and temperature (B) on % bindings of afl B1 (Y), while incubation time (C) at the center point, are shown in Fig. 1a. Fig. 1b shows effect of pH (A) and incubation time (C) on % bindings of afl B1 (Y), while temperature (B) at the center point. Fig. 1c shows effect of temperature (B) and incubation time (C) on % bindings of afl B1 (Y), while pH (A) at the center point. Corresponding to these plots, pH, time and temperature had a relevant effect on % bindings of afl B1. In studies where the incubation time was kept at the central point, the % binding of afl B1 on toxin binder increased at high temperature and pH values.

The present study aimed to determine quickly the optimum % bindings of afl B1 with fewer experiments. For this reason, desirability function (D) was used for determining the optimum experimental conditions (Fig. 2). The desirability function (D) can be used to deal with the optimization of multiple response problems in which too many variables are affected. The desirable ranges according to the goal can be changed from zero to one or target value. The target value was set at maximize for % bindings of afl B1. Desirability values (optimum experimental conditions) of maximize for % bindings of afl B1 were found to be pH 5.8, temperature 42°C and incubation time 94.11 min by using desirability function. In the study which was carried out under optimum experimental conditions, % bindings of afl B1 was found to be 97%.

Many articles have been published about the binding of afl B1 in chicken feeds on inorganic toxin binders such as active clinoptilolite and bentonite and organic toxin binders such as glucomannan and yeast (Diaz et al., 2003; Faucet-Marquis et al., 2014; Bočarov-Stančić et al., 2018; Yalcin et al., 2018; Ahn et al., 2022). These studies are usually focused on single toxin binding studies. The mix binder including clinoptilolite and yeast was used in our study. While the binding capacity of zeolite containing clinoptilolite was 21.9 % in the study performed by Vekiru et al. (2015), the binding capacity of zeolite was found to be 95.5% in a study conducted by Bočarov-Stančić et al. (2018). Faucet-Marquis et al. (2014) and Ahn et al. (2022) found that max % bindings of afl B1 were 70% and 92.3%, respectively, in the studies in which yeast was used as binder. However, in our study where a mixture binder containing clinoptilolite and yeast was used, the maximum max % bindings of afl B1 was found to be 97%. The reason for the higher % bindings of afl B1 is thought to be due to the synergistic effect of both organic yeast and inorganic clinoptilolite binders.

4. Conclusions

In this study, % binding capacity of afla B1 of mixed toxin binder used as toxin binder in poultry nutritions was evaluated using central composite design technique. pH, temperature and incubation time were used as variables in the binding capacity experiments and it was observed that these variables they had a significant effect on afl B1 binding when taking into consideration to 3D plots. With the help of the proposed quadratic model, ANOVA, desirability function and 3D graphics, it was found that the optimum experimental conditions for the highest % binding capacity of afl B1 were pH 5.8, temperature 42°C and incubation time 94.11 min. It was observed that the mixed toxin binder binds 97% afl B1 when optimum experimental conditions were used. It was found that mixed toxin binder had binding of afl B1 at a high rate. As a conclusion, it has been seen that the CCD can be used effectively in determining the toxin binding capacity of toxin binders.

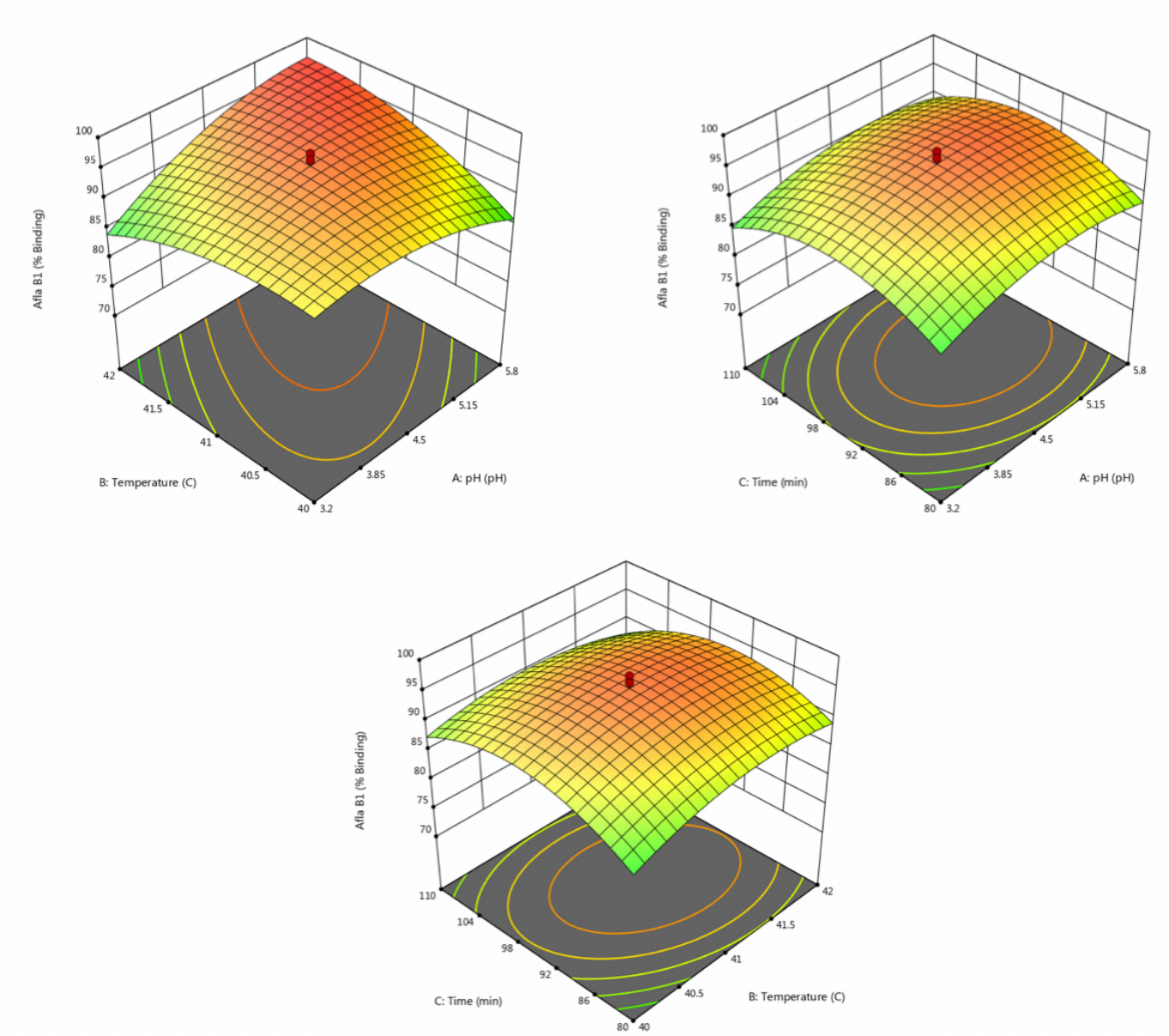

Fig. 1. The three-dimension response surface plots (3D) and normal plot of the residuals of % Binding (Y), A: pH; B: Temperature (°C); C: Incubation Time (min).

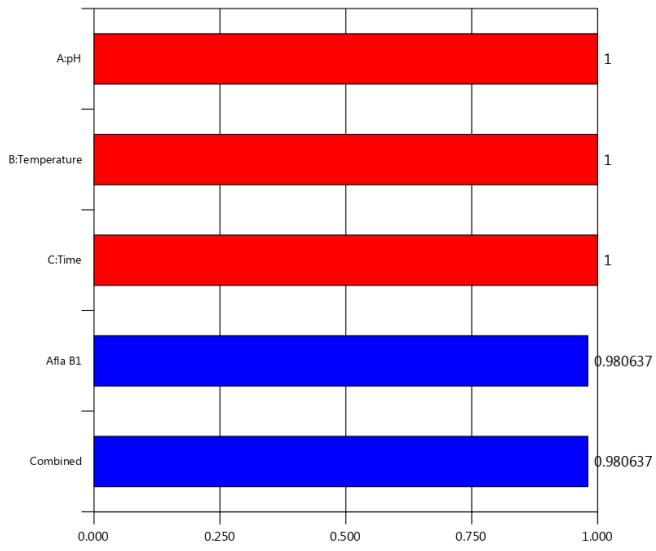

Fig. 2. Desirability bar graph.

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CRediT author statement

NFY, CC and MT conceived and HU and STHS supervised this study. NFY, CC and MT completed the main experimental content. NFY and CC and MT collected and analyzed data. MT and MSA wrote the first draft of the article. All authors contributed to the critical revision of the article and have read and approved the final version.

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