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Modelling approach to limit aflatoxin B_1 contamination in dairy cattle compound feed

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RESEARCH ARTICLE

Abstract

Feeding dairy cattle with safe compound feed helps farmers to ensure food safety. However, several ingredients often used in compound feed production can be contaminated with aflatoxin B_1 (AFB_1), which may result into milk contaminated with aflatoxin M_1. Given the number of ingredients and their amounts used in the production of compound feed, it is very costly to check every batch of ingredients for AFB_1 contamination. Which is the reason, why a risk-based approach is taken in the latest years. This study aimed to estimate the probability of AFB_1 contamination of compound feed for dairy cattle, and to limit this contamination, by optimisation of the compound feed formulation, using a modelling approach. The modelling approach comprised integrating a linear optimisation programming model to a Monte Carlo simulation model. This model was applied to the case of producing compound feed for dairy cattle in the Netherlands, using national monitoring data on AFB_1 contamination in feed materials collected in the period 2000-2010. Results from this case study showed the model can be used to produce safe compound feed with the lowest possible probability of AFB_1 contamination.

Keywords: mycotoxins, linear programming, Monte Carlo simulation, feed ingredients

1. Introduction

Several feed ingredients and the resulting feed produced thereof, such as compound feed, may be contaminated with mycotoxins. The consumption of mycotoxin contaminated feed has detrimental effects on animal health, including feed rejection, poor feed conversion, reduced body weight gain and reproductive performance, and increased disease frequency (Voss *et al.*, 2007). Aflatoxins, in particular aflatoxin B_1 (AFB_1), are of the most important mycotoxins known today. These toxins can cause liver cancer and have been implicated in child growth impairment and acute toxicoses (Wild and Gong, 2010; Wu *et al.*, 2014). In this respect, contamination of dairy cattle feed with AFB_1 is of concern, given the presence of this toxin in crops used for cattle feed production, and the transfer of this toxin into milk, where it appears as aflatoxin M_1 (AFM_1). AFB_1 and AFM_1 are genotoxic, carcinogenic and teratogenic for both animals and humans (Milićević *et al.*, 2010). The International Agency for Research on Cancer (IARC) has classified AFB_1 as an agent carcinogenic to humans (Group 1) and AFM_1 as an agent possibly carcinogenic to humans (Group 2B). Given the toxicity of AFB_1 and AFM_1, the European Commission (EC) has adopted regulations to limit the presence of both these aflatoxins in food (EC, 2006) and of AFB_1 in feed (EC, 2002) in Europe. The maximum level limit of AFB_1 is set at 20 μg/kg for feed ingredients, and at 5 μg/kg for compound feed for dairy cattle.

Until about a decade ago, AFB_1 has mainly been found in feed ingredients (maize, rice, sunflower seeds, etc.) originating from countries with tropical weather conditions, like India, Brazil, and Colombia, favourable for the presence of the responsible *Aspergillus* spp. (Bryden, 2012; Milićević *et al.*, 2010; Reddy *et al.*, 2009). However, during the latest decennia, AFB_1 has also been reported to occur in high concentrations in crops, especially maize, cultivated in Europe. For instance, in 2003, a hot and dry season resulted into severe contamination of maize grown in Northern Italy (Piva *et al.*, 2006). This maize had already been used as feed for dairy cattle and the result was a widespread contamination of AFM_1 in milk, exceeding the EC legal limit (being 0.05 μg/kg). In 2013, maize originating from the Balkan, was found to be contaminated with AFB_1, after import to Germany and the Netherlands. Part of this maize had already been fed to dairy cattle, and elevated levels of AFM_1 in milk had been reported (De Rijk *et al.*, 2015).

The presence of aflatoxins in feed and food materials needs to be checked in order to ensure the proper functioning of prevention and control measures in place, and to ensure concentrations are well below legal limits. Given the costs for sampling and analyses, samples are nowadays collected from feed ingredients that have the highest probability of contamination (so-called risk-based monitoring) and, consequently, several ingredients lots are not checked. In addition, some contaminated feed ingredient lots can wrongly be classified as acceptable (De Rijk *et al.*, 2015; Whitaker, 2003), because of sampling strategy variability and heterogeneity of aflatoxin contamination of ingredient lots.

This study aimed to estimate the probability of AFB_1 contamination of compound feed for dairy cattle, and to limit this contamination, by optimisation of the compound feed formulation, using a modelling approach. The approach was demonstrated to the production of compound feed for dairy cattle in the Netherlands.

2. Materials and methods

A modelling approach was developed to determine the optimised composition of compound feed for dairy cattle, i.e. with the AFB_1 concentration aimed to be below the EC legal limit (5 μg/kg), given the AFB_1 distributions in feed ingredients used in the formulation. The model contains two main steps: (1) optimisation of the composition of the compound feed, given a number of constraints, and (2) simulation to analyse the AFB_1 contamination probability in dairy cattle compound feed (Figure 1). The model was applied to AFB_1 contamination in compound feed for dairy cattle in the Netherlands, using national monitoring data on AFB_1 contamination in feed ingredients.

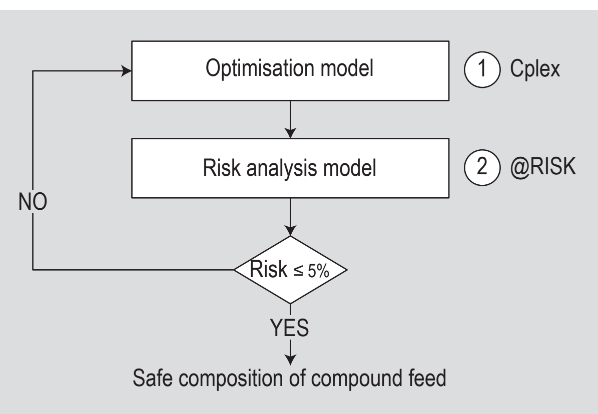

Figure 1. Schematic presentation of the modelling approach.

In step 1, the most important feed ingredients used in the formulation of compound feed for dairy cattle had been identified. The distribution of AFB_1 contamination in each of these ingredients was defined using SPSS.22 software (SPSS, Chicago, IL, USA) and the available monitoring data. Then, the composition of compound feed was optimised using a linear programming model developed on Cplex.12 solver (IBM, Armonk, NY, USA). In step 2, a risk analysis model was developed to estimate the distribution of AFB_1 contamination in compound feed based on Monte Carlo simulation. The following subsections describe in detail these two steps.

Optimisation model

Data processing

For the aims of this study, national monitoring data on AFB_1 concentrations in feed ingredients were subtracted from the database of the Quality of Agricultural Products (KAP) program, which is hosted by the National Institute for Public Health and the Environment (RIVM) in Bilthoven in the Netherlands. This database contains the results of the national monitoring program for chemical contaminants in feed materials in the Netherlands. For compound feed production for dairy cattle in the Netherlands, samples are collected from feed materials at different stages of the production chain, from home-produced and imported feed ingredients to the final compound feed.

Data from all feed ingredients analysed for the presence of AFB_1 in the period 2000-2010 were used, covering in total 9,523 records (monitoring results of one sample of an unique batch). When the analytical result was recorded as below the limit of detection (LOD) of 1 μg/kg of the method used, it was recorded as a zero in the database. Private industry from the Netherlands was contacted to retrieve a representative formulation, i.e. set of ingredients and their rate of inclusion, for producing compound feed for dairy cattle. The recipe and the inclusion rate of each ingredient used in the compound feed were validated based on the study of Devun *et al.* (2014). These authors reported the general composition of compound feed for cattle to be: 20.5% of all cereals, 18% co-product cereals, 10% citrus pulp, 2% beet pulp, 15% soy bean meal, 0.5% vegetable fat, 23% sunflower seed meal, 8% alfalfa and 3% for minerals.

Linear programming model

A Linear programming (LP) model (1)-(7) was developed to minimise production costs and the concentration of AFB_1 in dairy cattle compound feed. The production constraints included in the model were based on literature (De Boever *et al.*, 1994) and the feed web site (http://www.feedipedia.org), being:

- the percentage of protein in 1 kg of compound feed is higher than 22%;
- the energy value in 1 kg of compound feed is higher than 11.89 MJ;
- the AFB_1 concentration in 1 kg of compound feed is less than 1.5 μg/kg;
- the percentage of cereals in 1 kg of compound feed is higher than 40%;
- the percentage of each of these ingredients (wheat, citrus pulp, and soy meal) in 1 kg of compound feed is less than 30%;
- the percentage of maize in 1 kg of compound feed is less than 40%;
- the percentage of cereals in 1 kg of compound feed is higher than 40%;
- the percentage of palm kernel expeller in 1 kg of compound feed is less than 22.5%;
- the percentage of minerals in 1 kg of compound feed is 5%;
- the percentage of sunflower seed oil in 1 kg of compound feed is 0.5%;
- the percentage of alfalfa in 1 kg of compound feed is 8%.

Table 1 presents the costs, protein and energy values of all ingredients used in this study.

The following parameters and variables are used in the LP model:

Parameters

c_i the price of 1 kg of ingredient *i* (euro);
α_i AFB_1 concentration in feed ingredient *i* (μg/kg);
γ_i gross energy value in 1 kg of feed ingredient *i* (MJ);
σ_i protein percentage in 1 kg of feed ingredient *i* (%);
β_S the upper limit of AFB_1 concentration in the compound feed (μg/kg); in this study, this target was set at 1.5 μg/kg;
β_E minimum limit of gross energy in 1 kg of compound feed (MJ);
β_p minimum limit of protein in 1 kg of compound feed (%);
β_c minimum limit for inclusion rate of cereals in 1 kg of compound feed (%);
β_w maximum limit for inclusion rate of wheat in 1 kg of compound feed (%);
β_m maximum limit for inclusion rate of maize in 1 kg of compound feed (%)
β_{cp} maximum limit for inclusion rate of citrus pulp in 1 kg of compound feed (%).

Decision variables

x_i the percentage of ingredient *i* in the compound feed (%).

The proposed LP model for the optimisation of the compound feed composition is presented below.

Objective function

The objective function (Equation 1) includes the costs for producing compound feed.

$$Min \sum_{i}^{n} c_i x_i \tag{1}$$

Constraints

Equation 2 ensures that the sum of inclusion rates of ingredients is equal to 1.

$$\sum_{i}^{n} x_i = 1 \tag{2}$$

Equation 3 puts an upper limit β_S on the concentration of AFB_1 in compound feed.

$$\sum_{i}^{n} \alpha_i x_i \leq \beta_S \tag{3}$$

Equation 4 puts a lower limit β_E for gross energy in 1 kg of compound feed.

$$\sum_{i}^{n} \gamma_i x_i \geq \beta_E \tag{4}$$

Table 1. Input values on costs, protein and gross energy, and maximum and minimum inclusion rates of all ingredients, as used in the Linear programming model.[1]

	MZ	PK	CP	WT	RB	SM	SSM	BP	SSO	AL	ML
Cost (€/kg)	0.2	0.157	0.213	0.123	0.235	0.44	0.25	0.125	0.762	0.151	0
Protein (% DM)	21.7	16.7	7	17.3	12.7	51.8	32.4	9.3	15.6	18.3	0
Gross energy (MJ/kg)	18.8	20.1	17.3	18.9	20.2	19.7	19.4	17	26.1	18	0
Maximum inclusion rate (%)	40	22.5	30	30	NL	30	NL	NL	NL	8	3
Minimum inclusion rate (%)	0	0	0	0	0	0	0	0	0	8	3

[1] MZ = maize, PK = palm kernel expeller, CP = citrus pulp, WT = wheat, RB = rice bran, SM = soy meal, SSM = sunflower seed meal, BP = beet pulp, SSO = sunflower seed oil, AL = alfalfa, ML = minerals, NL = no limit.

Equation 5 puts a lower limit β_p for protein in 1 kg of compound feed.

$$\sum_{i}^{n} \sigma_i x_i \geq \beta_p \tag{5}$$

Equation 6 puts a lower limit β_c for cereals in 1 kg of compound feed.

$$\sum_{i}^{m} x_i \geq \beta_c \tag{6}$$

Equation 7 defines the maximum percentage of wheat used in 1 kg of compound feed.

$$x_i \leq \beta_w \quad i = w \tag{7}$$

Equation 8 defines the maximum percentage of maize used in 1 kg of compound feed.

$$x_i \leq \beta_m \qquad i = m \tag{8}$$

Equation 9 defines the maximum percentage of citrus pulp used in 1 kg of compound feed.

$$x_i \leq \beta_{cp} \qquad i = cp \tag{9}$$

Finally, Equation 10 defines the types of the variables being used.

$$x_i \geq 0 \qquad \forall\, i \tag{10}$$

Simulation model

The simulation model aimed to estimate the concentration ranges of AFB_1 in dairy cattle compound feed. The model was developed using @RISK 6 software (Palisade Corporation, Ithaca, NY, USA) (Figure 2). @RISK uses Monte Carlo simulation to identify, measure, and root out the causes of variability in a system. For each run, 1000 iterations were done. A sensitivity analysis was conducted to identify ingredients that had the highest influence on the probability of compound feed AFB_1 contamination. The focus of the sensitivity analysis was on the ingredients with the (apparent) highest AFB_1 concentration, being sun flower seeds, rice bran, wheat and maize.

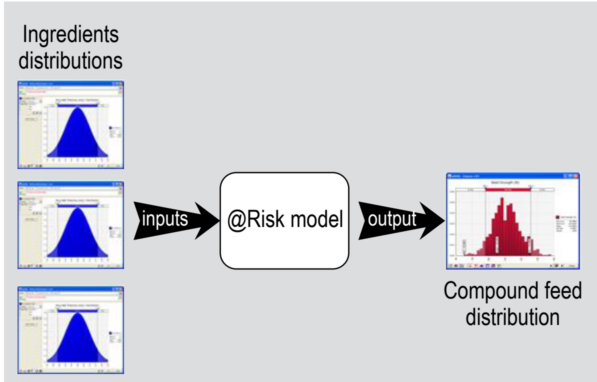

Figure 2. Illustration of the simulation model.

3. Results and discussion

Aflatoxin B_1 contamination in feed ingredients

The distribution of AFB_1 in the majority of feed ingredients was best described by a log-normal distribution. For example, the distribution of AFB_1 in maize is described by a log-normal distribution with mean 0.65 µg/kg and a standard deviation of 5.51 µg/kg (Table 2). In contrast, concentrations in ingredients such as beet pulp, sun flower seed oil, alfalfa and minerals were best described by a constant value equal to 0 µg/kg (Table 2).

The concentrations of AFB_1 in the feed ingredients used for dairy compound feed production in the Netherlands (Table 2) seem somewhat lower than the concentrations reported in earlier studies. Monbaliu *et al.* (2010) analysed a total of 82 feed samples, covering sow feed (n=4), wheat (n=30), and maize (n=48), collected from several EU countries (Czech Republic, Denmark, Hungary, Spain, and Portugal) and found that AFB_1 concentrations ranged 36-5,114 µg/kg. In a global survey, results of 11,967 samples of different feed ingredients and compound feeds that were analysed for the sum of aflatoxins B_1, B_2, G_1 and G_2 between 2005

Table 2. Aflatoxin B_1 concentration of feed ingredients (distribution, average, and standard deviation).

Ingredients[1]	MZ	PK	CP	WT	RB	SM	SSM	BP	SSO	AL	ML
Distribution[2]	Log-N	Log-N	Log-N	Log-N	Log-N	Log-N	Log-N	Const	Const	Const	Const
Average (µg/kg)	0.65	0.53	0.14	0.09	13.64	0.04	18.03	0	0	0	0
Standard deviation (µg/kg)	5.51	3.15	1.4	1.03	20.3	0.24	55.3	0	0	0	0

[1] MZ = maize, PK = palm kernel expeller, CP = citrus pulp, WT = wheat, RB = rice bran, SM = soy meal, SSM = sunflower seed meal, BP = beet pulp, SSO = sunflower seed oil, AL = alfalfa, ML = minerals.
[2] Log-N = Log normal distribution; Const = Constant.

and 2012 were evaluated (Schatzmayr and Streit, 2013). The number of positive samples was 3,124 (26%), and the average concentration in the positive samples was 57 μg/kg (median 11 μg/kg, and 3rd quartile 40 μg/kg). Results are only presented for the total of the four aflatoxins, not for AFB_1 separately. However, it is known that the aflatoxins B_2, G_1 and G_2 often co-occur with AFB_1 but in much lower concentrations. High concentrations of aflatoxins were found in feed and feed ingredients sources from South and South-East Asia (Schatzmayr and Streit, 2013). Dutch feed producers do not source much from this area, which could partly explain the difference in contamination data.

Optimised composition of compound feeds

The optimised compound feed composition, obtained from solving the LP model, is shown in Table 3. Feed ingredients used in the optimised composition include: maize (29.4%), palm kernel expeller (22.5%), wheat (30%), soy meal (5.1%), sunflower seed meal (1.5%), sunflower seed oil (0.5%), alfalfa (8%), and minerals (3%). Thus, the ingredients that are used most are wheat, maize and palm kernel expeller. Wheat is the cheapest ingredient (Table 1, 0.12 €/kg) and has a low average AFB_1 concentration (Table 2, 0.09 μg/kg), according to the current data used, and optimisation results showed this ingredient was used at its maximal inclusion rate of 30%. Besides data on costs and AFB_1 concentration of the ingredients used to solve the model, the optimised compound feed composition also depends on the requirements of dairy cattle compound feed for protein and energy, and for cereals and minerals in the compound feed. In practice the list of ingredients that could be used in compound feed production for dairy cattle is much longer than the ingredients considered in the current study, and also these ingredients have certain (minimal and/or maximal) inclusion rates in the compound feed. The current model included the most important ingredients, but can also be extended to included other ingredients that are often used in much lower inclusion rates.

Probability of exceeding the aflatoxin B_1 legal limit

Figure 3 presents the AFB_1 distribution and the probability of exceeding the EC legal limit in two compound feed compositions: the general composition proposed in literature, and the optimised safe composition (presented in Table 3). In case of the general composition (Figure 3A), the probability that AFB_1 concentration in compound

Table 3. Resulting optimised composition of dairy cattle compound feed compared to the general composition (%) (Devun *et al.*, 2014).

Ingredients[1]	MZ	PK	CP	WT	RB	SM	SSM	BP	SSO	AL	ML
Optimised safe composition	29.4	22.5	–	30	–	5.1	1.5	–	0.5	8	3
General composition (Devun *et al.*, 2014)	20.5	16	10	2	5	10	23	2	0.5	8	3

[1] MZ = maize, PK = palm kernel expeller, CP = citrus pulp, WT = wheat, RB = rice bran, SM = soy meal, SSM = sunflower seed meal, BP = beet pulp, SSO = sunflower seed oil, AL = alfalfa, ML = minerals.

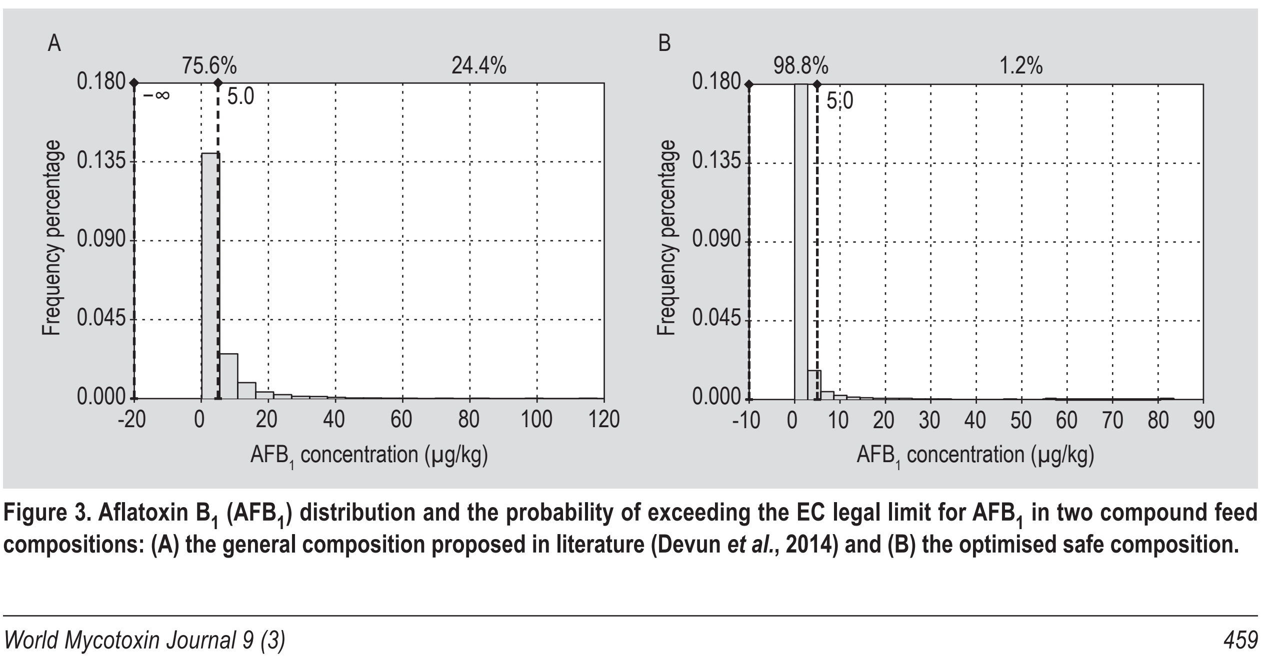

Figure 3. Aflatoxin B_1 (AFB_1) distribution and the probability of exceeding the EC legal limit for AFB_1 in two compound feed compositions: (A) the general composition proposed in literature (Devun *et al.*, 2014) and (B) the optimised safe composition.

feed exceeds the EC legal limit is 24.4%, the average AFB_1 concentration is 5.03 μg/kg, with a standard deviation of 10.9 μg/kg. Similar results were observed in Portugal (Martins *et al.*, 2007), where 37.4% of samples of feed for dairy cattle were AFB_1 positive (>1 μg/kg). AFB_1 contamination above the EC legal limit (>5 μg/kg) for dairy cattle feed were observed in 62 samples (6.2%), with concentrations ranging from 5.1-74 μg/kg.

Figure 3 shows the decrease in the probability of exceeding the legal limit of AFB_1 in compound feed from 24.4 (Figure 3A) to only 1.2% (Figure 3B) when the optimised feed composition is used. In this case the average AFB_1 concentration is 0.6 μg/kg with a standard deviation of 1.4 μg/kg. It is evident from Figure 3 that the use of the optimisation model would increase the safety of the compound feed for dairy cattle by reducing the probability of exceeding the EC legal limit for AFB_1.

Sensitivity analysis

Sunflower seeds inclusion rate

To analyse the impact of sunflower seed meal on the probability of contamination of compound feed with AFB_1, the rate to which this ingredient was used in the formulation was increased from 0 to 35%. Correspondingly, the rate for wheat use was reduced from 25 to 2%, and the rate for palm kernel expeller was reduced from 16 to 4% (Figure 4). The use of sunflower seed meal increases the probability of exceeding the EC legal limit of AFB_1 in compound feed for dairy cattle. As shown in Figure 4, increasing the inclusion rate of sunflower seeds in the compound feed formulation from 0 to 35% results into an increase of the probability of AFB_1 contamination of the compound feed from 1 to 32%. The safe inclusion rates (contamination probability <5%) are presented in scenarios 1 to 3.

Rice bran inclusion rate

Results for the effects of the inclusion rate of rice bran on the probability of exceeding the EC legal limit of AFB_1 in compound feed for dairy cattle are shown in Figure 5. The probability of contamination with AFB_1 is high in all scenarios. Increasing the rate of rice bran from 5 to 35% and reducing the rate of palm kernel from 16 to 0% increases the probability of contamination from 25 to 54%. It can be seen from Figure 5 that the inclusion rate of rice bran in the feed formulation had a marked effect on the probability of contamination of the compound feed. The probability for AFB_1 contamination of the compound feed increases with an increasing rate of rice bran. The effects for palm kernel and maize are opposite; thus, replacing feed ingredients that have a low probability to be contaminated with AFB_1, such as palm kernel and maize, in the feed formulation with an ingredient that has a high probability to be contamination, such as rice bran, will almost linearly increase the probability of AFB_1 contamination of the resulting compound feed.

Wheat inclusion rate

The effect of changes the rate of wheat in the formulation on AFB_1 contamination probability in compound feed is shown in Figure 6. Both wheat and sun flower seeds were observed to have a significant impact on AFB_1 contamination probability. It can be seen that replacing sun flower seeds by wheat reduces the probability of contamination. Reducing the rate of sun flower seeds from 23 to 2%, and replacing it by wheat from 2 to 27% decreases the contamination probability to less than 1%. The probability of contamination of the compound feed is below 5% when the inclusion rate of sunflower seeds is less than 6% and the inclusion rate of wheat is higher than 23%.

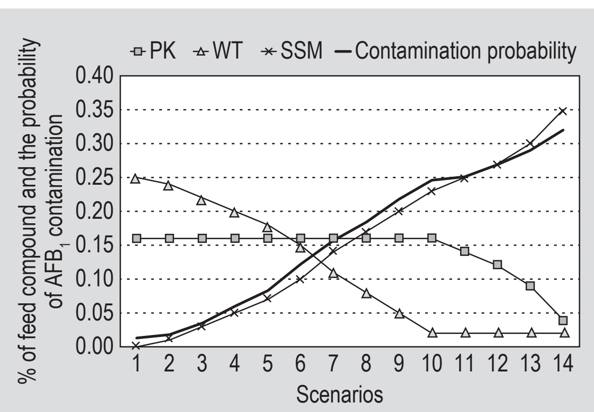

Figure 4. The impact of sunflower seed on the probability of exceeding the EC legal limit for aflatoxin B_1 (AFB_1) of compound feed for dairy cattle. PK = palm kernel expeller; WT = wheat; SSM = sunflower seed meal.

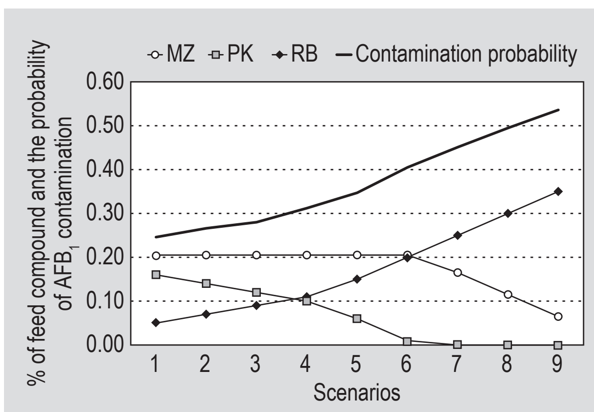

Figure 5. The impact of rice bran on the probability of exceeding the legal limit for aflatoxin B_1 (AFB_1) of compound feed for dairy cattle. MZ = maize; PK = palm kernel expeller; RB = rice bran.

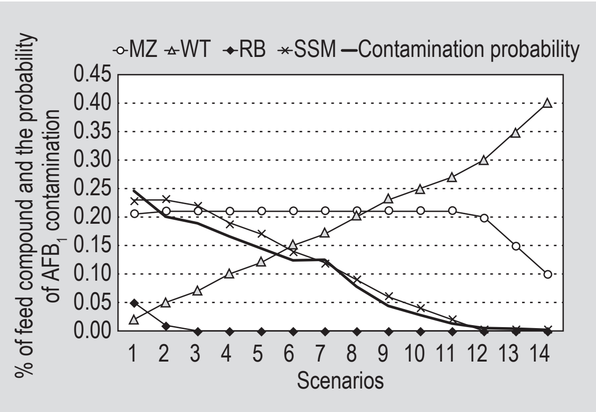

Figure 6. The impact of wheat on the probability of exceeding the legal limit for aflatoxin B_1 (AFB_1) in compound feed for dairy cattle. MZ = maize; WT = wheat; RB = rice bran; SSM = sunflower seed meal.

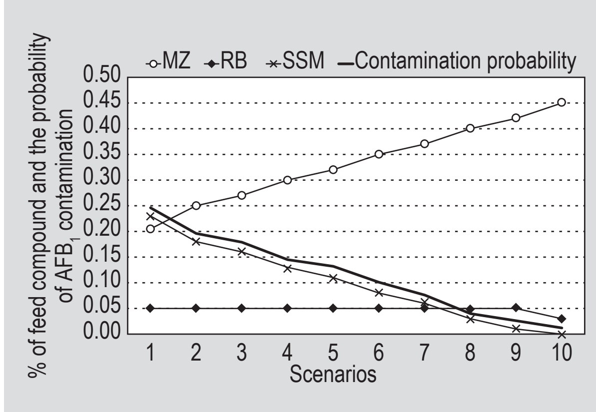

Figure 7. The impact of maize on the probability of exceeding the legal limit for aflatoxin B_1 (AFB_1) in compound feed for dairy cattle. MZ = maize; RB = rice bran; SSM = sunflower seed meal.

Many safe formulations are proposed in Figure 6 (scenario 9 to scenario 14).

Effect of maize percentage variation

The results of the variation of maize percentage in compound feed formulation are shown in Figure 7. Both maize and sun flower seeds had a significant impact on the probability of exceeding the legal limit of AFB_1 in compound feed. From Figure 7, it can be seen that replacing sun flower seeds by maize reduces the probability of contamination. Decreasing the rate of sun flower seeds from 23 to 0% and replacing it by maize from 21 to 45% reduces the contamination probability from 25 to 1%. This is due to the reduction of sunflower seeds used in the compound feed composition, which is directly correlated to the contamination probability.

Wheat price

The optimised safe composition is sensitive to the price of the ingredients, i.e. when the prices change the optimised composition of the compound feed will also change (Table 4). When the price of wheat increases from 0.12 to 0.4€, the inclusion rate of wheat in the compound feed decreases from 30 to 0%. Figure 8 shows the variation in feed wheat prices between 2012-2015. Results showed that when the wheat price rises from 0.12 to 0.18€, the inclusion rate of wheat decreases from 30 to 24%, and wheat is replaced by maize. When the wheat price is between 0.2 and 0.3€, only 3% of wheat is used, and 17% of beet pulp is used, together with more soy meal and maize. When the wheat price is equal to or higher than 0.4€, wheat is fully replaced by maize (40%), palm kernel (5%), soy meal (11%), and beet pulp (32%).

Table 4. Effect of wheat price on the optimised compound feed composition.[1]

Wheat prices (€)	MZ	PK	CP	WT	RB	SM	SSM	BP	SSO	AL	ML	Contamination probability
0.12	0.29	0.22	-	0.30	-	0.05	0.02	-	-	0.08	0.03	0.02
0.15	0.29	0.22	-	0.30	-	0.05	0.02	-	-	0.08	0.03	0.02
0.18	0.37	0.22	-	0.24	-	0.05	-	-	-	0.08	0.03	0.01
0.20	0.37	0.22	-	0.03	-	0.09	-	0.17	-	0.08	0.03	0.01
0.25	0.37	0.22	-	0.03	-	0.09	-	0.17	-	0.08	0.03	0.01
0.30	0.37	0.22	-	0.03	-	0.09	-	0.17	-	0.08	0.03	0.01
0.40	0.40	0.05	-	-	-	0.11	-	0.32	-	0.08	0.03	0.01

[1] MZ = maize, PK = palm kernel expeller, CP = citrus pulp, WT = wheat, RB = rice bran, SM = soy meal, SSM = sunflower seed meal, BP = beet pulp, SSO = sunflower seed oil, AL = alfalfa, ML = minerals.

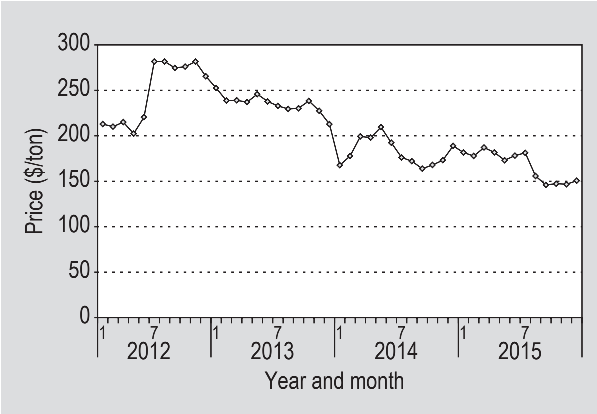

Figure 8. Feed wheat prices from 2012 to 2015.

General discussion

This study developed a modelling approach to estimate the probability of AFB_1 contamination of compound feed for dairy cattle, and to limit this contamination by optimising the inclusion rates of the different feed ingredients. The model can be added as a module to current optimisation programs for compound feed composition applied by feed mills. Currently, such optimisation programs account for prices of ingredients and nutritional requirements of the particular animal category for which the compound feed is produced, as well as some other constraints, such as the quality of the compound feed. When the current modelling approach will be added, also the aflatoxin contamination of feed ingredients is accounted for, in such a way the final product, the compound feed, has an aflatoxin concentration that is a low as possible or below a certain pre-defined threshold. Adding to management practices already in place to limit aflatoxin contamination in feed ingredients and in the final product, such as monitoring of ingredients, this module will add an additional safeguard tool for compound feed producers. It cannot and will never replace current sampling and analyses programs. However, would a particular contaminated batch accidentally be missed in current monitoring programs, this module can still ensure feed safety. Given the heterogeneity of aflatoxin contamination of batches of ingredients (De Rijk *et al.*, 2015), declaring a batch of feed ingredient as having a contamination below the EC legal limit, while it is actually above ('false-negative'), can occur, even with very intensive monitoring.

The optimised compound feed composition depends on the data used to solve the model such as: the ingredients costs, the AFB_1 concentration of each ingredient, and requirements of dairy cattle regarding the amounts of protein, energy, cereals, and minerals in the compound feed. The current dataset used showed that aflatoxin AFB_1 concentrations in the feed ingredients were relatively low, as compared to available literature. Since the samples were collected from feed ingredients sourced by Dutch feed mills from all over the world, the feed mills probably are already able to avoid highly contaminated batches of ingredients. When early information or signals for contamination of certain ingredients are available, the expected contamination could be used, rather than historical data. When weather information is available, it might even be possible to use predictions from mycotoxin forecasting models (Van der Fels-Klerx *et al.*, 2010), e.g. for aflatoxins in maize as data input, provided such models are validated and have shown their correctness for predictions.

In the current model version, only some major constraints for nutritional requirements were considered, such as the minimal amount of energy and protein needed. In reality, much more detailed requirements for e.g. presence of certain amino acids are known per animal category, and accounted for in compound feed composition. Such more detailed nutritional requirements can be added to the program, and will then be considered as additional model constraints.

Application of the model to AFB_1 in compound feed for dairy cattle, using the current dataset and constrains, showed that the optimised safe composition (lowest probability of contamination) includes: maize (29%), palm kernel (23%), wheat (30%), soy meal (5.1%), sunflower seed meal (1.5%), sunflower seed oil (0.5%), alfalfa (8%) and minerals (3%). This composition coincides quite well with dairy cattle feed formulations found used in practice about a decennia ago in the Netherlands (Van Raamsdonk *et al.*, 2007). Results demonstrated that it is possible to lower the use of sun flower seed meal and rice bran as ingredients (having highest AFB_1 contamination probability) such to reduce the probability of exceeding the AFB_1 legal limit in compound feed. Variation in prices of feed ingredients will also impact the compound feed composition. For example, when the price of wheat increases, the wheat is replaced by cheaper alternatives impacting AFB_1 contamination. Data on the use of feed ingredients in Great Britain in 2013 (Johnson, 2014) showed that the increase of the wheat price in 2013 (0.3$/kg) resulted in an increase in the amount of maize feed used (+107.7%) and a decrease in the use of wheat feed (-9.2%), other cereals (-7.8%), citrus and other fruit pulp (-64.4%), dried sugar beet pulp (-11.8%), and rice bran extractions (-32.7%).

The model is very flexible regarding the animal category and the mycotoxin of concern. For instance, other toxins can be added to the current module, such to account for the maximal presence of two different mycotoxins at the same time. Although the presence of other mycotoxins than aflatoxins, are currently not regulated by the EC, guidance values do exist for several mycotoxins, such as deoxynivalenol, zearalenone and ochratoxin, in products intended for animal feeding (EC, 2006b). Dairy cows (Fink-

Gremmels, 2008), amongst other animals, are sensitive to different mycotoxins, so putting constraints on the maximal presence of each of them, would be even better. The current application for compound feed composition for dairy cows does not account for possible exposure of dairy cows to aflatoxins via roughage or silage at the farm, since it is focused at the optimal compound feed composition. It is however, known that silage and roughage may also contribute to mycotoxin load of dairy cattle, though these are not a major source for aflatoxins.

The model can also easily be adapted for another toxin, such as deoxynivalenol and compound feed composition for another animal, e.g. sows or breeding pigs, by adapting the model constraints, and the dataset used. The model can support risk managers in feed industry. Feed mills can use their own historical data on mycotoxin contamination and prices, as well as their own constraints for nutritional requirements, such to adapt the model to their use.

4. Conclusions and recommendations

We proposed a modelling approach to minimise the possible contamination of AFB_1 in compound feed through optimising the feed formulation, considering the EC legal limit for the presence of AFB_1 as one of the constraints in the model. This modelling approach could be used by feed industry to extent their current decision tools and algorithms for compound feed composition. When the EC legal limits for AFB_1 contamination for compound feed would be integrated in their current optimisation of the use of feed ingredients, this would further contribute to the production of safe compound feed. The model can never replace current sampling and analysis. However, in addition to current monitoring programs in place at the private feed industry and the government, it would be an additional step in safeguarding the production of safe compound feed. The model is very flexible, and can easily be adapted for application to other mycotoxins and/or compounds feeds, such as to deoxynivalenol in compound feed for pigs. Also, several others dairy cattle requirements in compound feed can be included as constraints in the model such as chemistry, digestion, and absorption of essential nutrients. In future work, additional uncertainty issues could be addressed and integrated in the model parameters, for instance for the prices of ingredients.

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