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Aflatoxin levels in commercial finisher balanced feeds for broilers and pigs from Venezuelan factories

Niveles de Aflatoxinas en alimentos finalizadores para pollos de engorde y cerdos procedentes de fábricas de alimentos balanceados comerciales en Venezuela

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ABSTRACT

Mycotoxins, even so government regulates to control their levels in animal balance feeds, they could pose a problem to animal and human consumption health. Therefore, the aim was to determine the levels of total aflatoxins found in the finisher balanced feeds for broilers and pigs, produced by balanced feed factories (BFF) in Venezuela. Eleven BFFs were sampled and evaluated for the presence of aflatoxins: AFB1, AFB2, AFG1 and AFG2, determined using the analytical chemical technique HPLC-FLD. Thirty three feed samples for broilers and 33 for pigs, a total of 66 samples were obtained from BFFs. The presence of aflatoxins was detected in 100 % of the finisher balanced feeds for poultry and pigs. The determined AFB1, AFB2, AFG1 and AFG2 levels in finisher balanced feeds for broilers and pigs, rendered that the aflatoxin with the highest concentration was always AFB1. The averages of total aflatoxins detected from finisher feeds for broilers and pigs were within the maximum allowed limits (20 micrograms (µg) / kilograms (kg) of COVENIN standards (Venezuelan regulations). When compared each aflatoxin level (AFB1, AFB2, AFG1 and AFG2) eight out of eleven BFFs evaluated comply with the standard, but 3 (F1, F2 and F5) BFFs were outside (5 µg/kg). It is pertinent that the companies that produce final food for poultry and pigs carry out additional and frequent evaluations of other mycotoxins (Ocratoxins, fusarium mycotoxins and mycotoxins emerging) present in raw materials, in order to evaluate the risks in the agri-food chain, in the way to implement solutions before or after processesment to ensure public health quality.

Key words: Aflatoxins; finisher feeds; broilers; pig; HPLC

RESUMEN

Las micotoxinas, aun cuando son reguladas por el Estado Venezolano para controlar sus niveles en los alimentos balanceados, podrían representar un problema para la salud del consumo animal y humano. El objetivo de la investigación fue determinar los niveles de aflatoxinas totales que se encuentran en los alimentos balanceados finalizadores para pollos de engorde y cerdos, elaborados por plantas productoras de alimento balanceado (PPAB) en Venezuela; se muestrearon once PPAB. Se evaluó la presencia de niveles de aflatoxinas: AFB1, AFB2, AFG1 y AFG2, utilizando la técnica química analítica HPLC – FLD. Con 33 muestras de alimentos para pollos de engorde y 33 para cerdos, para un total de 66 muestras. Se detectó la presencia de aflatoxinas en el 100 % de los alimentos balanceados finalizadores para aves y cerdos evaluados. Los niveles de AFB1, AFB2, AFG1 y AFG2, en alimentos balanceados finalizadores para pollos de engorde y cerdos, se encontró que la aflatoxina en mayor concentración resultó ser siempre la B1. Los promedios de aflatoxinas totales detectadas, de alimentos finalizadores para pollos de engorde y cerdos estuvieron dentro de los límites máximos (20 microgramos (µg) / kilogramos (kg)) permitidos (normas COVENIN). Al realizar la comparación entre niveles de aflatoxinas (AFB1, AFB2, AFG1 y AFG2), ocho de once PPAB evaluadas cumplen con la norma, y 3 (F1, F3 y F5) están fuera de ella (5 µg/kg). Es pertinente que las empresas productoras de alimentos finalizadores para aves y cerdos realicen evaluaciones de otras micotoxinas (ocratoxinas, fusarium micotoxinas y micotoxinas emergentes) presentes en las materias primas y evaluar los riesgos en la cadena agroalimentaria, para así implementar soluciones antes y después del procesamiento para garantizar la calidad de la salud pública.

Palabras clave: Aflatoxinas; alimentos finalizadores; pollo de engorde; cerdos; HPLC

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INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO) [33], up to 25 % of food crops worldwide are contaminated with a type of mycotoxin. Among them, aflatoxins have been reported, and defined as secondary metabolites produced by strains of fungi of the genus *Aspergillus*, being generally aflatoxin B1, a highly hepatotoxic compound in poultry and pigs production, as carcinogenic in pigs and humans [19, 25, 34, 42]. They are frequently detected in seeds or vegetable raw materials [16, 22], are thermostable and the pelletization process of animal feed will not destroy them, if made with contaminated raw materials [44]. Therefore, presence of mycotoxins in food is considered a safety feeding issue, and only maximum limits are allowed locally in Venezuela [13, 14] or internationally [18].

Venezuela as a tropical Country is not protected from these types of mycotoxins and their toxic effects. They are present in different raw materials used in the production of balanced foods [9, 11, 16, 26], where manufacturing factories (F) located mainly at the central region of the Country, within the States of Aragua, Carabobo and Cojedes, as the Western State of Zulia, constitute the main poultry producers. The previous, with the North of Guárico and Miranda States have the highest intensive production of pigs (*Sus scrofa domestica*)[1, 35].

In view of high production of balanced feed and due to its great demand within the poultry (*Gallus gallus domesticus*) or pig production sector, an attempt has been made to protect human and animal health from the harmful and agro-economic effects of aflatoxins [6, 25, 28, 40, 47]. Agrovigilance organizations in Venezuela through the COVENIN Standards [14], established that total aflatoxins or other contaminating substances should not be in levels higher than 20 µg/kg food formulations [14, 36]. However, in Venezuela there is limited scientific information regarding the amount of aflatoxins that could be present in the balanced feed consumed by pigs and broilers at their finishing stage. To them being benefited at the agri-food chain, which represents a public health risk and threatens the safety of these food products, by not being certain of the contamination levels of this mycotoxin in these type of food formulations.

As previously described, the research purpose intend to determine the levels of total aflatoxins (AFB1, AFB2, AFG1, AFG2), which are found in the finisher balanced feeds for broilers and pigs, processed and marketed by feed-producing F in Venezuela.

MATERIALS AND METHODS

Locations

The collection and assessment of samples were carried out in a laboratory specialized in toxicology, located in Maracay, Aragua State, Venezuela (Latitude 10°15'06" N | Longitude 67°36'05" O). The environmental conditions that the laboratory had were: average temperatures of 30 °C with an average relative humidity of 40 %, of course all analysis were performed over enviromental controlled conditions.

Methodologic approach

The trial period was 6 months, from January to June 2010, where 3 randomize samples of finisher feed for broilers and 3 for pigs in the finishing stage were collected and evaluated at the laboratory, procedent from each factory (F). A total of 11 Balanced Food Factories (BFF) were subjected to assessment. Therefore, 33 BFF samples were generated for broilers and 33 for pigs, a total of sixty-six (66) samples. As they were arriving, the aflatoxin extraction method, AOAC 2005-991 [7], was carried out in order to preserve the extracted aflatoxin under refrigerated (Revco Scientific. Inc. ULT 1386-3-A14, EUA) conditions (4°C) for later use in the following detection technique, AOAC 2005.08-2005 [8].

In all cases, the quality control personnel of the companies or F carried out the primary and random sampling, directly from their production process, according to COVENIN 1567:80 standard animal feed, sampled method refered before [45]. These samples were homogenized and subsampled until obtaining a 1 kg sample, seeking to obtain a representative subsample of the sampling. Samples were delivered to the specialized toxicology laboratory that would carry out the collection and evaluation of samples.

Sample procedure for samples total aflatoxin determination

Chromatographic techniques were used for the extraction of aflatoxins, the official method AOAC 2005, 991.31 was applied [7]. A 25 grams (g) sample was placed in a blender, with 5 g of NaCl plus 125 mililiters (mL) of methanol: water (7:3). Mixed at high speed (mixer Vortex, MIX, Argolab, Spain) for 2 minutes (min). Subsequently, was filtered with 24 centimeters (cm) Whatman #2 paper. A 15 mL of the filtered solution was pipetted into a 125 mL balloon. Then, 30 mL of water were added, mixed and filtered with GF/A 9.0cm glass microfiber paper. Following, a 15 mL of the second filtrate was transferred through the immunoaffinity column [28], washed with 10 mL of distilled water. Finally, the aflatoxin was extracted with 2.0 mL of High Performance Liquid Chromatography (HPLC) extracted methanol and placed in a 2.0 mL vial.

To determine aflatoxin levels, the official AOAC 2005, 2005.08 method was used [8], in a reversed phase HPLC chromatograph coupled to a model 2475 fluorescence detector (HPLC-FLD Agilent 1100, Hewlett Packard, USA). The method is based on the fact that the aflatoxins B1, B2, G1 and G2 show natural fluorescence, although those type B1 and G1 have little fluorescence, therefore, an attached Kobra Cell post-column electrochemical cell a (KOBRA® CELL R-BIOPHARM RHONE Ltd, EUA), to HPLC was used, where the sample reacted with an derivatizer agent (potassium bromide salt), so that aflatoxins B1 and G1 were derivatized to their bromide derivatives, which gave enhanced fluorescence. It should be noted that the use of the Kobra Cell, apart from significantly increasing the fluorescence of aflatoxins B1 and G1, allows the detection and quantification of less than 0.5 µg/kg for each of the four individual toxins [43].

The eluate obtained was evaporated and reconstituted with 2.0 mL of mobile phase. With a flow of 1.0 mL/min of mobile phase, 20 microliters (µL) of standard and sample were injected. For the determination of the detection limit and quantification of the HPLC-FLD technique, 20 µL of total aflatoxin standard was injected at different concentrations: 1.0, 1.25, 1.5, 10 and 20 µg/kg. It should be noted that these indicators were used in order to determine and/or verify the reliability of measurements obtained from the aflatoxins levels. The previous was according to the HPLC-FLD-electrochemical cell method, which has a detection and quantification limit that is less than 2 µg/kg of total aflatoxin or less than 0.5 µg/kg for each individual aflatoxin (B1, B2, G1, G2). The detection limits: 1 µg/kg of total aflatoxin, and quantification: 1.25 µg/kg of total aflatoxin of the HPLC-FLD technique were established.

According to the values obtained for the detection limit, the minimum reliably detectable quantity was 1 µg/kg of total aflatoxin, which is less than 0.5 µg/kg (0.25 µg/kg) when the four individual toxins are accounted for, it was possible to verify the validity of the applied technique, when evaluated foods were analyzed, since it matches with those declared by the methodology (less than 0.5 µg/kg for each of the four individual toxins). In the same way, it was fulfilled for the quantification limit, where the minimum amount of the analyte quantifiable was 1.25 µg/kg of total aflatoxin (0.31 µg/kg for each of the four individual toxins). The validity of the HPLC-FLD technique could be verified by evaluating aflatoxins in feeding products for broilers and pigs at the finalizing production stage.

Statistical analysis

Descriptive statistics was used to characterize the variables under study. Likewise, the experiment was carried out under a randomized design. Model Type 1: Balanced Fixed Unifactorial Effects. The basic assumptions were tested and unviable for the variables under study, therefore the non-parametric test of Kruskal Wallis by ranks under a one-way analysis of variance [38] was performed and with a degree of significance equal to 0.05. Evaluation was applied to each type of feed: poultry and pigs and its data were analyzed with the statistical package Statistix Vers. 8.0.

RESULTS AND DISCUSSION

Eleven Venezuelan food F were evaluated, putting emphasis that the maximum allowed limit for total aflatoxin is 20 µg/kg [14] and 5 µg/kg [13] for each type of aflatoxin (AFB1, AFB2, AFG1 and AFG2), established by COVENIN standards (Venezuelan Standards), which allowed the comparison of obtained results.

Additionally, it should be appointed that COVENIN regulation it is currently at revision and to get publish with modification although it is not available, nevertheless there should be point that most of the feeding factories investigated are following current guidelines of animal feeding [14]. F have their own internal quality control of the production proccess over primary cereal sources, with stronger level restrictions, such as, the established at the food for human consumption in Venezuela [13].

The total aflatoxins average concentration for each F under study did not exceed the maximum allowed limit, although aflatoxins were present in all samples obtained. The results from the aflatoxin levels were the following: AFB1, AFB2, AFG1 and AFG2, which were present in finisher balanced feeds for broilers and pigs. Aflatoxin levels and statistic evaluations were shown at TABLES I-VI.

In TABLE I, all eleven F presented Aflatoxin B1, being the most toxic of this group. Where AFB1 had an average value of 2.95 µg/kg among the 11 F, with a mean standard error of 0.86, the lowest value of 0.31 µg/kg and the higher of 9.15 µg/kg, followed by aflatoxin B2 and G1. The lowest concentration of aflatoxin was on G2, with an average value of 0.66 µg/kg, the lowest of 0 µg/kg and the highest of 1.83 µg/kg. On the other hand, it should be noted that the F with the highest level of aflatoxins B1 was F1, with a value of 9.15 µg/kg.

At analysis of these results, aflatoxin levels: AFB1, AFB2, AFG1 and AFG2 in the evaluated feeds samplings, AFB1 was present at all samples, where two evaluated F (F1 = 9.15 µg/kg and F3 = 6.97 µg/kg) were outside the maximum allowed limits (5 µg/kg), within a total of 18.18 %.

TABLE I
Results of the descriptive statistics for 11 Venezuelan balanced feed factories, when evaluating Aflatoxin levels: AFB1, AFB2, AFG1 and AFG2, present in finisher balanced feeds for Broilers

FACTORY	Aflatoxin levels (µg/kg)			
	B1	B2	G1	G2
F1	9.15	5.28	2.57	0
F2	1.82	3.80	2.35	1.83
F3	6.97	1.68	1.63	1.30
F4	4.19	1.70	0.31	0.31
F5	0.78	1.58	0.48	0.31
F6	0.63	0.77	0.00	0.00
F7	0.31	0	0	0
F8	1.00	0.42	0.47	0.32
F9	3.98	2.67	0.52	0
F10	1.85	1.75	1.83	1.75
F11	1.78	1.57	1.92	1.45
Average	2.95	1.93	1.10	0.66
SEM	0.86	0.46	0.29	0.23
Lowest	0.31	0	0	0
Higher	9.15	5.28	2.57	1.83

SEM: Standard Error Mean

It should be noted that F1 also is outside maximum limits allowed in AFB2 (F1 = 5.28 µg/kg), corresponding to 9.09 % of the total. Although the aforementioned F were outside the limit established by the COVENIN standard, the average of the eleven F did not exceed 5 µg/kg for each type of aflatoxin.

The previous data confirms that it was possible to obtain the total aflatoxin levels for each F under study, presented in TABLE II, which describes the statistics of the total aflatoxin levels found in the finisher balanced feed samples for broilers.

TABLE II, in the case of balanced feeds for broilers in the finishing stage, it can be noted that all the reported evaluated feeds had presence of total aflatoxins. Finding as an average level in eleven F evaluated the amount of 6.65 µg/kg, with an average standard error of 2.28 µg/kg, a minimum value of zero (0) µg/kg and a maximum value of 29.07 µg/kg. In turn, it can be observed that the F that reports the highest average concentration of total aflatoxins is F1 with 17.0 µg/kg, followed by F3 (11.58 µg/kg) and F2 (9.8 µg/kg). The lowest level of total aflatoxins corresponded to F7 (0.37 µg/kg).

The Kruskal-Wallis test was significant ($P<0.05$), when compared concentrations of total aflatoxins in the eleven studied F. The test of means reflects in TABLE III that F1 to F2 were homogeneous, F10 to F5 were homogeneous with each other. The F8 and F6 were also homogeneous, unlike the F1 it is different from the F7.

TABLE II
Results of the descriptive statistics when evaluating the levels of total Aflatoxin in samples of finisher balanced feeds for Broilers from eleven factories of balanced feeds in Venezuela

FACTORY	Average concentrations of total aflatoxin (µg/kg)	SEM	Lower (µg/kg)	Higher (µg/kg)
F1	17.00	6.58	6.43	29.07
F2	9.80	3.58	4.60	16.65
F3	11.58	4.27	3.36	17.70
F4	6.51	2.46	1.60	9.92
F5	3.15	1.05	1.83	5.23
F6	1.4	0.74	0	2.40
F7	0.37	0.23	0	0.80
F8	2.21	1.27	0.93	4.75
F9	7.17	3.54	0.65	12.84
F10	7.18	0.26	6.85	7.70
F11	6.72	1.07	5.10	8.75
Average	6.65	2.28		
Lower	0.37		0	
Higher	17.00			29.07

SEM: Standard Error Mean

TABLE III
Range means test obtained when evaluating total Aflatoxin levels in samples of finisher balanced feeds for Broilers from eleven feed factories in Venezuela

FACTORY	Average concentrations of total aflatoxin (µg/kg)
F1	17.00 ^{a} ± 0.58
F3	11.58 ^{ab} ± 4.27
F2	9.80 ^{abc} ± 3.58
F10	7.18 ^{bcd} ± 0.26
F9	7.17 ^{bcd} ± 3.54
F11	6.72 ^{bcd} ± 1.07
F4	6.51 ^{bcd} ± 2.46
F5	3.15 ^{bcd} ± 1.05
F8	2.21 ^{cd} ± 1.27
F6	1.40 ^{cd} ± 0.74
F7	0.37 ^{d} ± 0.23

Different letter within the same column denote significant differences = a - d. n = 3, P<0.05, Standard Error Mean to compare = 4.2098

The data in TABLE IV reflects that all the eleven F feeds present all types of aflatoxins (AFB1, AFB2, AFG1 and AFG2). Where the highest level in AFB1, with an average value of 3.45 µg/kg, a mean standard error of 1.0, a minimum value of 0.58 µg/kg and a maximum value of 10.53 µg/kg, followed by AFB2 and AFG2. The lowest average concentration was AFG1 with 1.3 µg/kg.

TABLE IV
Results of the descriptive statistics by balanced feed processing factories, at evaluated levels of Aflatoxins: AFB1, AFB2, AFG1 AND AFG2, in finisher feeds for Pigs in Venezuela

FACTORY	Aflatoxin level (µg/kg)			
	B1	B2	G1	G2
F1	9.19	2.93	0.65	0
F2	1.68	2.93	1.59	0.87
F3	10.53	2.87	1.60	1.57
F4	3.98	0.31	0	0
F5	2.81	5.63	0.42	0.45
F6	0.69	0.95	0	0
F7	1.6	1.68	1.48	1.47
F8	0.58	2.72	2.82	3.49
F9	1.97	2.50	2.05	2.13
F10	1.88	1.87	2.00	3.27
F11	3	3.27	2.17	2.31
Average	3.45	2.51	1.34	1.41
SEM	1	0.42	0.28	0.39
Lower	0.58	0.31	0	0
Higher	10.53	5.63	2.82	3.49

SEM: Standard Error Mean

TABLE V presents the total aflatoxin levels in pig finishers feed for each F sampled, it can be seen that all the evaluated feeds samples reported on average the presence of total aflatoxins. Finding as the average level of aflatoxins in the total F a value of 8.72 µg/kg, associated with a mean standard error of 1.92 with a minimum level of 0.8 µg/kg and a maximum level of 27.2 µg/kg.

Similarly, it can be seen that the F that reports the highest total aflatoxin level is F3 (16.57 µg/kg), followed by F1 (12.77 µg/kg). Meanwhile, the F with the lowest level of aflatoxins is F6 (1.64 µg/kg).

The Kruskal-Wallis test was not significant (P>0.05) when comparing the concentrations of total aflatoxins in the eleven F investigated. The results of the range means tests were reflected in TABLE VI, indicating that there were no significant differences between the eleven F, although F3 is different from F6 (P<0.05).

TABLE V
Results of the descriptive statistics to evaluate the levels of Total Aflatoxin, in samples of balanced feeds for Pigs in the finishing stage, from eleven feed factories in Venezuela

FACTORY	Average concentrations of total aflatoxin (µg/kg)	SEM	Lower (µg/kg)	Higher (µg/kg)
F1	12.77	7.21	5.39	27.20
F2	7.07	1.25	4.76	9.06
F3	16.57	2.55	12.00	20.80
F4	4.29	3.28	0.80	11.27
F5	9.31	0.76	7.90	10.52
F6	1.64	0.44	2.10	2.60
F7	6.23	0.24	5.95	6.70
F8	9.61	1.55	7.02	12.40
F9	8.65	0.55	7.55	9.20
F10	9.02	1.17	7.45	11.30
F11	10.75	2.63	7.72	15.95
Average	8.72	1.92		
Lower	1.64		0.80	
Higher	16.57			27.2

SEM: Standard Error Mean

TABLE VI
Range means test obtained when evaluating total Aflatoxin levels in balanced feed samples for Pigs in the finishing stage and from eleven feed factories in Venezuela

FACTORY	Average concentration of total aflatoxin (µg/kg)
F3	16.57 ^{a} ± 2.55
F1	12.77 ^{ab} ± 7.21
F11	10.75 ^{ab} ± 2.63
F8	9.61 ^{ab} ± 1.55
F5	9.31 ^{ab} ± 0.76
F10	9.02 ^{ab} ± 1.17
F9	8.65 ^{ab} ± 0.55
F2	7.07 ^{ab} ± 1.25
F7	6.23 ^{ab} ± 0.24
F4	4.29 ^{ab} ± 3.28
F6	1.64 ^{b} ± 0.44

Different letter within the same column denote significant differences = a - d. n = 3, P<0.05, Standard Error Mean to compare = 3.8587

Comparison with the maximum allowed limits (COVENIN standard) and total aflatoxin levels, detected in finisher feed for broilers (33 samples) and finisher feed for pigs (33 samples), were shown in TABLES VII and VIII.

TABLE VII shows that there is no important difference in the results of finisher feeds for broilers compared to pigs. Both previous cases present 18.18 % of positive samples (above 5 µg/kg), in AFB1 compared to the total (eleven F evaluated) and 9.09 % in AFB2. The levels of AFG1 and AFG2 were kept below 5 µg/kg (maximum limit allowed by COVENIN), for this reason the average and the percentage of positive samples with concentration above the maximum allowed level were not assigned in TABLE VII.

TABLE VIII shows that the balanced feeds for finisher pigs, from eleven F evaluated presented slightly higher levels of total aflatoxin (8.72 ± 1.92 µg/kg) than the balanced feed for broilers (6.65 ± 2.28 µg/kg), and that both types of feeding do not exceed the maximum limits established by the National standard in their average total aflatoxins.

On the other hand, it should be noted that the F with the highest level of AFB1 (with higher toxicity of the group), is F3 (10.53 µg/kg), followed by F1 (9.19 µg/kg), these were equivalent at 18.18 % of samples outside the maximum allowed limits (5 µg/kg). The one with the highest concentration in AFB2 is in F5 (5.63 µg/kg), equivalent to 9.09 %.

However, when observing the maximum concentration values, it is distinguished that they are higher than the maximum allowed levels, in finisher feeds for broilers and pigs (TABLE VIII) in F1 samples. Likewise, in the detected values, 2 of the F in the case of broilers (F1 and F3), and three in the case of pigs feeding (F1, F3 and F5), are outside the limits established by the Venezuelan standard (COVENIN) by its levels in AFB1 and AFB2 (5 µg/kg).

These results can be attributed to the inadequate handling of raw materials or the poor control of biotic and abiotic factors that favor the colonization of cereals by aflatoxigenic strains, such the followed conditions: humidity, temperature, oxygen and the constitution of the substrate at the time of drying and/or the storage of cereal raw materials, regional socioeconomic condition, among other intervening variables that are described by several authors [6, 9, 11, 26, 28, 44]. Without forgetting that the random distribution of mycotoxins affects in this sense, even if all F buy or are sourced from the same supplier [27].

However, the data from the present research also indicates that nine of the Venezuelan BFF sampled are carrying out quality controls, both for raw materials and food, in accordance with national and international regulations. Possibly emphasizing the good selection of raw materials. One could even assume the quality of the genetic content that they possess. In this regard, it should be mentioned that the Venezuelan BFFs, in order to meet the demand for balanced food production, they must consider current regulations, the little or no availability of some raw materials for agroclimatic or socioeconomic reasons, among others. Having to resort to importation, as is the case for corn (*Zea mays*) and soybeans (*Glycine max*), so they do not escape the use of transgenic cereals and oilseeds, in the production of their products [10, 28, 48].

It has been shown that in Bt maize hybrids, there is a reduction of the vulnerability of grains to fungi that produce mycotoxins compared to non-transformed hybrids, especially for the control of maize spoilage caused by *Aspergillus* and *Fusarium* genus [37, 48], perhaps decreasing the aflatoxigenic strains of fungi in cereal crops. However, at the time of sampling in this research, there was no legislation on

TABLE VII
Comparison of Aflatoxin levels in finisher balanced feeds for Broilers and Pigs, from eleven food factories in Venezuela

Toxins	Broiler				Pig			
	R	O	X̄	M %	R	O	X̄	M %
AFB1	0.31 - 9.15	2/11	8.06	18.18	0.58 - 10.5	2/11	9.86	18.18
AFB2	0 - 5.28	1/11	5.28	9.09	0.31 - 5.63	1/11	5.63	9.09
AFG1	0 - 2.57	0/11	-	-	0 - 2.82	0/11	-	-
AFG2	0 - 1.83	0/11	-	-	0 - 3.49	0/11	-	-

AFB1= Aflatoxin B1; AFB2= Aflatoxin B2; AFG1= Aflatoxin G1; AFG2= Aflatoxin G2. R= aflatoxin concentration ranges (lower and higher µg/kg). O = Occurrence of positive samples/total plants sampled. X̄ = average concentration of positive samples (µg/kg). M % = percentage of positive samples with concentration above the maximum allowed level (5 µg/kg allowed COVENIN standard).

TABLE VIII
Averages of total Aflatoxins in samples of finisher balanced feeds for Broilers and Pigs from eleven factories in Venezuela

Balanced feed	Average of total Aflatoxins (µg/kg)	Higher value (µg/kg)	Higher allowed limits of total Aflatoxins (µg/kg)
Broilers	6.65 ± 2.28	29.07 (F1)	20
Pigs	8.72 ± 1.92	27.2 (F1)	20

F1: balanced feed factory where the maximum values were detected

the production and import of transgenics products in the Country, so imports of transgenic corn and soybeans from Countries, such as the United States of America (USA), Brazil and Argentina, were probably carried out [10, 37, 48].

This previous idea could contribute to justify the low levels of aflatoxins detected in this research and that most of the BFF sampled are within the allowed levels of national regulation. Additionally, the detected values ratify the results obtained in similar investigations and samplings on dates close to the present evaluation [28], where aflatoxin levels were evaluated in finisher feeds for pigs, and did not exceed the Venezuelan regulations in farms of the Center States of Aragua and Carabobo. Likewise, in this previous research similar reasons are discussed to explain the aflatoxin levels found in the present study. Additionally, total aflatoxin levels similar to those of the present research, but actually higher than those allowed (greater than 20 µg/kg), were detected in feed for broilers, obtained from feeders in Venezuelan poultry farms as previously described [6].

Research carried out in poultry have determined that low concentrations of aflatoxins due to periods of prolonged exposure generally report subclinical effects and not for that reason, less harmful, which frequently require to be demonstrated by very sensitive liver tests or examinations that allow detecting the true severity of aflatoxicosis [4, 5, 15, 31]. These situations can also be similar/translated to pigs and other monogastric species.

Additionally, experiments in broilers that received levels of 20 µg/kg of aflatoxin B1 in their diet for 5 weeks, suggested a tendency for the duodenum of these broilers to be more susceptible to infection by *Eimeria acervulina* [29]. A prolonged exposure with low concentrations of aflatoxins in their diet causes important immunosuppressive effects, characterized by the unexpected appearance of infectious and parasitic diseases, in addition the animals do not respond adequately to vaccination programs [32, 41], these immunotoxic effects will increase susceptibility to infectious and contagious diseases, with low concentrations of aflatoxins in the diet have also been described in pigs [39, 40, 42, 47].

Other reports, internationally, there was an analysis (chromatographic methods) of the natural presence of mycotoxins (enniatins, beauvericin, ochratoxin A, aflatoxins, alternariol monomethyl ether, alternariol, tentoxin, zearalenone, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyl-2 and HT-2), from 122 samples of balanced feeds marketed in Tunisia, which were destined for poultry (n = 43), found that poultry feeds were among the most contaminated by other mycotoxins, levels of aflatoxins that did not exceded the established limits by the European Union in animal feed [24]. Likewise, to evaluate (analytical chemical methods) mycotoxin contamination in Nigeria, 102 samples of balanced feed (n = 30) and their raw materials (n = 72) were collected, from poultry farm mills in 12 states, where the most common mycotoxin in balanced feed was fumonisin B1, and aflatoxin B1, which was detected in 83 % of the feed samples at a concentration of 74 µg/kg, where feed samples analyzed in this study were contaminated with at least four mycotoxins: aflatoxins and fumonisins coexistent in 80 % of the samples [2].

Other authors in South America, have evaluated mycotoxins (analytical chromatography method) in samples of corn and finished poultry feeds produced in Brazil, in 119 samples, collected from farms with balanced feed for poultry: Most of the samples were contaminated with more than one mycotoxin, where the mean aflatoxin and trichothecene contamination was low, close to the allowed values [30].

Others in Europe, collected 228 pig feed samples in Spain to detect (by analytical chromatography) 19 mycotoxins (aflatoxins B1, B2, G1 and G2, ochratoxin A, fumonisins B1 and B2, citrinin, zearalenone, deoxynivalenol, fusarenon X, sterigmatocystin, T2, HT-2 toxin, enniatins A, A1, B, B2 and beauvericin). Most of the samples (96.9 %) agreed with the European Union regulations (which do

not address emerging mycotoxins: enniatin B and beauvericin, or co-occurrence), the authors highlight that their results show that for to ensure absence of mycotoxins, emerging mycotoxins should always be considered [3].

Likewise, this study also allows to see the need to evaluate the probable synergistic risks, due to interactions between different mycotoxins, in the presence of low levels of aflatoxins, despite the fact that their concentrations are within the regulated limits. However, although in the present study one hundred percent (100 %) of feeds sampled, both for poultry and pigs, presented some type of aflatoxin (TABLES VII and VIII), no levels were found that could cause human or animal deaths. However, despite the sublethal levels of aflatoxins in this study, it should be noted that these official maximum allowed limits (20 µg/kg) are based on avoiding the presence of aflatoxin residues in foods of animal origin for human consumption. Taking the human species as the "most susceptible link" to aflatoxins within the food chain for reasons of agri-food safety and public health [6]. In the same way, is to say, there is no safe value of aflatoxins due to their cumulative effect [12, 20, 46], so even when tolerable levels are established for the animal and even values achieved are low, it must be taken into account that the meat of these animals will be consumed by the human population [21]. Threading to a toxicity risk for humans that should be avoid by stricter limits, and better supervisión by the authorities.

Therefore, it needs to be consider the risk levels of aflatoxin residues that are in the agri-food chain, which do not depend exclusively over the aflatoxin levels in the feeding finisher level, but will be present in the animal product to human comsuption.

Meaning that the probability of other intervening variables should also be considered, such as the case of repeated improvements in animal genetic lines, which modify the consumption and food conversion in the animals [17], and/or their metabolic rate, being able to promote that broilers and pigs, eventually have different toxicokinetics from those expected or usual for aflatoxins in farms. This would depend on several factors to which the broilers or pigs are exposed, before reaching the slaughterhouse.

Such factors can be extreme management, or interactions with: nutrients, additives, other mycotoxins, disinfectants, drugs, agrochemicals and other xenobiotics to which the animals are exposed. The previous with low concentrations of aflatoxins in the diet, could generate an interaction effect framed within the toxicology principle known as additivity and synergism.

Additivity occurs for example when the combined effect of two mycotoxins is equal to the sum of the effect of each mycotoxin supplied individually. Synergism occurs, when the total effect of two mycotoxins is greater than the sum of their individual effects and the synergistic interaction causes the greatest toxic effects, as has been observed between aflatoxin with ochratoxin A or toxin T-2 [23]. Despite being in low concentrations or at permitted levels in the feed of animals destined for human consumption, it is not easy to predict the possible interactions and their level of residues or harmfulness for the agri-food chain, when there are low concentrations of aflatoxins and other mycotoxins at varying levels or unknown.

CONCLUSIONS

In the 11 Venezuelan balanced feed manufacturing F sampled, during 2010, the presence of aflatoxins was detected in 100 % of the final balanced feed for poultry and pigs. At the levels of AFB1, AFB2, AFG1 and AFG2, in finisher balanced feeds for broilers and pigs was found that the aflatoxin in the highest concentration was always B1, considered the most toxic of the group.

When compared the averages of total aflatoxin concentrations, from samples of 11 Venezuelan F., which produced finisher balanced feeds for broilers and pigs, with maximum limits (20 µg/kg) allowed (COVENIN standards), it turned out that standards were followed. However, when comparing the levels by type of aflatoxins AFB1, AFB2, AFG1 and AFG2, it is established that by type the maximum allowed limit is 5 µg/kg, where 8/11 F. evaluated comply with the standard, and three (F1, F3 and F5) were out of the allowed limits.

RECOMMENDATIONS

Monitoring of the genetic quality of raw materials, and requirements of the supplier to declare the certificated quality of transgenic or not (currently it is not declared), in order to correlate with studies such as the one in this evaluation.

It is pertinent that the companies that produce finishing foods for the final production stage of poultry and pigs carry out evaluations not only in terms of aflatoxins, but also of other mycotoxins present in raw materials of transgenic and non-transgenic cereals, and evaluate the probable agri-food chain risks according to the present results.

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CONFLICTS OF INTEREST

The authors declare no conflicts at the present research.

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