







Aflatoxins and fumonisins in feed from a broiler operation system from São Paulo state, Brazil

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ABSTRACT: *The aim of the present study was to assess the occurrence of aflatoxins (AFs) and fumonisins (FBs) in feed ingredients (corn and soybean meal) and finishing feed in a broiler operation system, as well as to evaluate their effect on the productivity of 20 batches of broilers produced and the histology status of broilers' liver after slaughter. Corn samples presented the highest frequencies of AFs and FBs, at mean levels of 29.1 and 2,100µg/kg, respectively. Soybean samples presented mean levels of 1.5 and 70µg/kg for AFs and FBs, respectively. Batches of broilers receiving feed containing FB levels higher than 1,000µg/kg had lower weight gain and higher mortality rates, while those fed rations with AFs equal or above the limit of quantification (LOQ) of the analytical method presented higher scores of histological changes in the liver. A dilution effect was observed for AFs and FBs from ingredients, especially corn, to feed during manufacture, whilst not enough to prevent losses in productivity. Results of this trial highlighted the need for strict control of mycotoxins in corn intended for broilers.*

Key words: *mycotoxins, AFB₁, FB₁, broiler chickens, productivity.*

Aflatoxinas e fumonisinas em rações de um sistema de produção de frangos de corte no estado de São Paulo, Brasil

RESUMO: *O objetivo do presente trabalho foi verificar a ocorrência de aflatoxinas (AFs) e fumonisinas (FBs) em ingredientes (milho e farelo de soja) e na ração de abate sobre a produtividade de uma empresa integradora de frangos de corte, bem como avaliar seus efeitos sobre produtividade de 20 lotes de frangos produzidos pela empresa e a histologia dos fígados dos frangos após o abate. As amostras de milho apresentaram as maiores frequências de AFs e FBs, em concentrações médias de 29,1 e 2.100µg/kg, respectivamente. As amostras de farelo de soja apresentaram níveis médios de 1,5 e 70µg/kg para AFs e FBs, respectivamente. Os lotes de aves que receberam ração contendo níveis de FBs maiores que 1,000µg/kg apresentaram menor ganho de peso e maior percentual de mortalidade, enquanto que as que receberam ração com AFs iguais ou superiores ao limite de quantificação (LQ) do método analítico apresentaram maior grau de alteração histopatológica no fígado. Houve efeito de diluição de AFs e FBs dos ingredientes, especialmente o milho, à ração no processo de fabricação, porém não suficiente para evitar perdas na produtividade. Os resultados do estudo reforçam a necessidade do controle estrito de micotoxinas no milho destinado à alimentação de frangos de corte.*

Palavras-chave: *micotoxinas, AFB₁, FB₁, frangos de corte, produtividade.*

INTRODUCTION

Mycotoxins are secondary metabolites produced by a range of toxigenic fungi that develop naturally in foodstuffs, which causes a great variety of toxic effects in several animal species (RICHARD, 2007). Broiler chicks are particularly sensitive to the adverse effects of mycotoxins in feed that was prepared with contaminated main ingredients, especially corn (OLIVEIRA et al., 2014). In Brazil, the most common toxigenic fungi reported in corn and corn-based feed included species from the genera *Aspergillus* and *Fusarium* (SANTURIO, 2000; AQUINO & POTENZA, 2013), which produce the

aflatoxins (AFs) and fumonisins (FBs), respectively. As a consequence, AFs and FBs are the most frequently reported mycotoxins in Brazilian corn and commercial feed for poultry (SOUZA et al., 2013). However, there is no regulation for mycotoxins in feed in Brazil, except for a recommended maximum value of 50µg/kg of AFs in feed ingredients (MINISTÉRIO DA AGRICULTURA, 1988). Soybean meal is considered a low-risk product for AF or FB contamination (OLIVEIRA et al., 2014); although, other *Fusarium* toxins such as nivalenol and deoxynivalenol have been reported (MARTINELLI et al., 2004). MALLMANN et al. (2001) did not observe any positive sample of soybean meal from

southern states in Brazil containing FBs, and there is no report on the occurrence of AFs in Brazilian soybean meal.

Twenty different types of AFs were identified; although, only AFB₁, AFB₂, AFG₁ and AFG₂ are frequently reported as natural contaminants of food products (HUSSEIN & BRASEL, 2001). Toxic effects of AFs include carcinogenicity, mutagenicity, teratogenicity and hepatotoxicity (JAGER et al., 2013). In poultry production systems, the AFs negatively affect the body weight gain and feed conversion, and causes histological changes in the liver as well as immunosuppression and increased mortality rates (RICHARD, 2007). Twenty-eight structurally related FBs have been isolated and identified, although FB₁, FB₂ and FB₃ are the most predominant form produced by the fungi (REISINGER et al., 2016). In poultry, FBs cause decreased feed consumption and body weight gain, increased relative weights of liver and kidney, and liver necrosis (HENRY et al., 2000; TESSARI et al., 2010).

The toxicological data available on the effects of AFs or FBs in broiler chickens are mostly based on experimental trials conducted at laboratory scale (ALLAMEH et al., 2005). However, there is no information on the health impacts of these mycotoxins under conditions of natural occurrence in the field, such as commercial poultry production units in Brazil. Thus, the present study aimed to determine the occurrence of AFs and FBs in the main feed ingredients (corn and soybean meal) and finishing feed of a broiler operation system, and evaluate their impact on the company's productivity.

MATERIALS AND METHODS

Broiler operation system characteristics and sampling procedures

The study was conducted in a broiler operation system located in the Southeast region of the State of São Paulo, which used the integration system composed by nearly 280 integrated farms to produce around 6,000 tons/month of finished products. The company produced the total feed required by the farms, using corn and soybean meal harvested in the Brazilian Mid-West region as main ingredients. In all integrated farms, the birds were vaccinated against Marek's disease (Fort Dogde®) and Gumboro disease (CEVAC® IBD L) at hatchery and at 14 day-old, respectively.

The following types of samples were collected in the study, during manufacture of 17 batches of feed: feed ingredients (corn and soybean

meal, N=17 for each product) received by the company and stored in the feed production plant, finishing feed freshly prepared (supplied to animals from 41 to 48 days of age) (N=47), and finishing feed stored (N=47) for up to 10 days in 7 farms with housing capacity ranging from 9,000 to 39,000 birds. Composed samples (2kg) were collected from of each product, and the number and weight of incremental samples collected from different points in the storage silos followed the recommendations of ISO 6497 (ISO, 2002). All samples were placed in polyethylene bags and sent to the laboratory for immediate analysis. The 17 batches of finishing feed were distributed to the 7 integrated farms of the company and used to produce 20 batches of broilers (N=4,000-5,000 birds per batch).

Analyses of aflatoxins and fumonisins

Extraction and purification of corn, soybean meal and feed samples for determination of AFs (AFB₁, AFB₂, AFG₁ and AFG₂) were performed using immuno affinity columns (Neogen®) as described by OLIVEIRA et al. (2008). For FBs (FB₁, FB₂), solid phase extraction (SPE) columns (Bond-Elut SAX) were used for extraction and purification according to BORDIN et al. (2014). The AFs and FBs were determined using a high performance liquid chromatography (HPLC) system, composed by a Shimadzu (Kyoto, Japan) 10VP liquid chromatograph, a 10 AXL fluorescence detector, a Shim-Pack CLC-ODS Sil column (4.6X250mm, 5µm) and a Shim-Pack pre-column (4X10mm, 5µm CLC G-ODS). Instrument set up, preparation of calibration curves, derivatization steps and chromatographic conditions for determination of AFs and FBs strictly followed the procedures as described by OLIVEIRA et al. (2008) and BORDIN et al. (2014). The retention times were approximately 4.2, 5.3, 7.6 and 9.5min. for AFG₁ (converted to AFG_{2a}), AFB₁ (converted to AFB_{2a}), AFG₂ and AFB₂, respectively. For FB₁ and FB₂, the retention times were approximately 9.5 minutes, respectively. The limits of detection (LOD) and limits of quantification (LOQ) were calculated based on signal: noise ratios of 3:1 and 10:1, respectively. The performances of the analytical methods were evaluated as previously described (OLIVEIRA et al., 2008; BORDIN et al., 2014).

Results reported in the finishing feed stored in the farms were categorized into four groups according to the levels of AFs (below and equal or above the LOQ value of the analytical method), and FBs (lower and equal or above 1,000µg/kg).

Productivity parameters

During the study, 20 batches of broilers were raised in the 7 integrated farms evaluated until the age of 48 days, when they were sent to the company's slaughterhouse. At the time of slaughter of broilers of each batch from each farm, the following parameters were calculated by the company: Average daily weight gain (g/day)=(final weight-initial weight)/period in days; Feed conversion (g/g)=Feed intake (g)/weight gain (g); Production factor=(viability x average daily weight gain)/(feed conversionx10); Mortality (%)=(number of birds at start - number of birds at the end)x100/number of birds at start.

Histological analysis

In the slaughterhouse, samples of liver, kidney, heart and bursa of Fabricius from 2 birds per batch of broilers were collected, totaling 40 samples of each organ. Samples were fixed in formalin, embedded in paraffin, sectioned at 4µm, and stained with hematoxylin and eosin stain for histopathology analysis (LUNA, 1968). Organs sections from all treatment groups were examined microscopically and individual sample numerical scores were reported using the following scoring system, based on the severity of the main mycotoxin-associated lesions (NEEFF et al., 2013): 0=Organ section unremarkable; 1=Lesions in organ section are compatible with mild mycotoxicosis, affecting less than 20% of the tissue; 2=Lesions in organ section are compatible with light mycotoxicosis, affecting 20% to 40% of the tissue; 3=Lesions in organ section are compatible with moderate mycotoxicosis, affecting 40% to 60% of the tissue; 4=Lesions in organ section

are compatible with serious mycotoxicosis, affecting 60% to 80% of the tissue; and 5=Lesions in organ section are compatible with severe mycotoxicosis, affecting more than 80% of the tissue.

Statistical analysis

The 20 batches of broilers produced in the 7 farms evaluated were classified in four categories, according to the AF or FB levels reported in the finishing feed stored in the farms, as described earlier. The productivity parameters and histopathological scores within each AF or FB category were analyzed by the Student's t-Test, using the statistical package SAS 9.1 (SAS, 2004). The differences among means were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The occurrence of AFs and FBs in samples of corn, soybean meal and finished feed (freshly prepared and stored in the farms) is presented in table 1. The frequency of samples containing quantifiable levels of AFs in corn samples (65%) was similar to the value reported (55%) in samples of freshly harvested maize in the State of São Paulo, with a highest concentration of AFB₁ of 1,600µg/kg (MACHINSKI et al., 2001). However, the mean value of total AFs for corn in the present study was 29.1±24.4µg/kg, which is below the recommended maximum value of 50µg/kg of total AFs in feed ingredients in Brazil (MINISTÉRIO DA AGRICULTURA, 1988). The highest AF levels was reported in a corn sample containing 115.3µg/kg, and this was the only sample with concentration of total AFs above the Brazilian

Table 1 – Occurrence of aflatoxins in samples of corn, soybean meal, finishing feed freshly prepared and finishing feed stored in integrated farms of a broiler operation in São Paulo, Brazil.

Type of sample	N	-----Total AFs ¹ -----		-----Total FBs ² -----	
		N (%)	Mean (µg/kg)	n (%)	Mean (µg/kg)
Corn	17	11 (65)	29.1±24.4	17 (100)	2,070±1,550
Soybean meal	17	1 (6)	1.5	10 (59)	70±30
Finishing feed (freshly prepared)	47	10 (21)	2.9±3.1	41 (87)	1,260±890
Finishing feed (stored in farm)	47	8 (17)	3.8±4.8	42 (89)	1,310±1,050

N: Number of samples analyzed.

n: number of samples with levels equal or above the limit of quantification (0.5µg/kg for each aflatoxin, and 30µg/kg for each fumonisin).

¹Sum of aflatoxins B₁, B₂, G₁ and G₂.

²Sum of fumonisins B₁ and B₂.

recommended limit. ROCHA et al. (2009) reported a lower frequency (10%) of freshly harvested corn contaminated with AF, at much higher levels (up to 1,906 $\mu\text{g}/\text{kg}$) than those observed in the present study.

The high frequency of FBs (100%) found in corn samples is consistent with previous studies demonstrating high occurrence rates of FBs in commercially available corn in Brazil (MORENO et al., 2009; ROCHA et al., 2009; QUEIROZ et al., 2012). In our study, the maximum concentration of total FBs was observed in a corn sample containing 3,900 $\mu\text{g}/\text{kg}$, which is lower than the highest level (6,450 $\mu\text{g}/\text{kg}$) reported by QUEIROZ et al. (2012) in corn from Minas Gerais State. Much higher FB levels (up to 18,740 $\mu\text{g}/\text{kg}$) were observed by VAN DER WESTHUIZEN et al. (2003) in corn from Santa Catarina State, at mean concentration 3,210 $\mu\text{g}/\text{kg}$. Soybean meal samples presented lower frequency (59%) and mean level (70 \pm 30 $\mu\text{g}/\text{kg}$) of total FBs.

Compared with corn, soybean meal samples had much lower frequency and levels of AFs or FBs than in corn, hence indicating its lower relevance as a source of mycotoxin contamination in the feed manufacture. MALLMANN et al. (2001) did not reported detectable levels of FBs in soybean meal samples from southern states in Brazil, which is in agreement with data reported here. Furthermore, samples of finishing feed also had lower AF or FB frequency and levels when compared with corn, which may suggest a dilution of the mycotoxin content from corn to feed during manufacture. ALLAMEH et al. (2005) also observed that using corn containing 1,000 $\mu\text{g}/\text{kg}$ of AFB₁ as ingredient resulted in the concentration of 650 $\mu\text{g}/\text{kg}$ in the feed. A similar reduction in the frequency of positive samples was reported in a study conducted in Egypt by ABDALLAH et al. (2017), who detected AFB₁

in 16% of corn and 4% of feed samples analyzed, at mean levels of 4.8 and 5.3 $\mu\text{g}/\text{kg}$, respectively. In our study, the frequencies and levels of AFs were similar in freshly prepared feed and feed available in the farms, thus indicating good storage conditions of feed in the farms evaluated. Our result for FBs is also in agreement with those reported by SOUZA et al. (2013), who observed median FB levels of 1,840 $\mu\text{g}/\text{kg}$ and 239 $\mu\text{g}/\text{kg}$ in samples of corn (before the factory processing) and poultry feed (after the factory processing), respectively. SOUZA et al. (2013) explained that the average contamination levels in poultry feed samples were lower due to the processing or the addition of other ingredients beside corn.

Productivity parameters of batches of broiler chicks categorized according to quantifiable levels of AFs (AFs<LOQ, AFs \geq LOQ) or FBs (FBs<1,000 $\mu\text{g}/\text{kg}$, FBs \geq 1,000 $\mu\text{g}/\text{kg}$) in the finishing feed stored in the farms are presented in table 2. No effect ($P>0.05$) was observed in the feed conversion and production factor of broiler categories. However, birds fed rations containing with FBs \geq 1,000 $\mu\text{g}/\text{kg}$ in the diet lower ($P<0.05$) weight gain (56.8 \pm 2.6g/day) and higher mortality rate (6.1 \pm 2.3%), when compared with the FBs<1,000 $\mu\text{g}/\text{kg}$ category. Birds receiving FBs \geq 1,000 $\mu\text{g}/\text{kg}$ in the diet had approximately 4% lower weight gain when compared to the FBs<1,000 $\mu\text{g}/\text{kg}$ group. RAUBER et al. (2013) observed that negative effects on performance of broilers receiving experimental feeds containing 100,000 $\mu\text{g}/\text{kg}$, which is much higher than the maximum FB level reported in the feed of the present study (3,950 $\mu\text{g}/\text{kg}$).

Table 3 presents the histopathological scores observed in the organs of batches of broiler chicks categorized according to LOQ for AFs or FBs in the finishing feed stored in the farms. No

Table 2 – Productivity parameters of 20 batches of broiler chicks categorized according to quantifiable levels of aflatoxins (AFs) or fumonisins (FBs) in the finishing feed stored in the farms.

Broiler batch category	Weight gain (g/day)	Feed conversion (g/g)	Production factor ¹	Mortality (%)
AFs<LOQ ² (N=12)	58.3 \pm 2.4 ^a	1.8 \pm 0.1 ^a	305.7 \pm 22.5 ^a	5.1 \pm 1.7 ^{ab}
AFs \geq LOQ (N=8)	57.7 \pm 3.0 ^{ab}	1.9 \pm 0.1 ^a	296.7 \pm 31.8 ^a	5.6 \pm 2.5 ^a
FBs<1,000 $\mu\text{g}/\text{kg}$ (N=10)	59.1 \pm 2.4 ^a	1.8 \pm 0.1 ^a	309.6 \pm 17.8 ^a	4.7 \pm 2.0 ^b
FBs \geq 1,000 $\mu\text{g}/\text{kg}$ (N=10)	56.8 \pm 2.6 ^b	1.9 \pm 0.1 ^a	291.1 \pm 34.1 ^a	6.1 \pm 2.3 ^a

^{a-b}Values within each column with no common superscript differ significantly ($P<0.05$).

¹Calculated as follows: Production factor=(viability x average daily weight gain)/(feed conversion x 10).

²LQ: Limit of quantification (0.5 $\mu\text{g}/\text{kg}$ for AFB₁, AFB₂, AFG₁ or AFG₂).

Table 3 – Histopathological scores observed in the organs of 20 batches of broiler chicks categorized according to quantifiable levels of aflatoxins (AFs) or fumonisins (FBs) in the finishing feed stored in the farms.

Lot category	Scores ¹			
	Liver	Kidney	Heart	Bursa
AFs<LQ ² (N=12)	0.3±0.1 ^b	0.1±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^a
AFs≥LQ (N=8)	1.2±0.2 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.2±0.1 ^a
FBs<1,000µg/kg (N=10)	0.3±0.1 ^b	0.2±0.1 ^a	0.2±0.1 ^a	0.3±0.2 ^a
FBs≥1,000µg/kg (N=10)	0.4±0.2 ^b	0.2±0.1 ^a	0.4±0.1 ^a	0.3±0.1 ^a

^{a-b}Values within each column with no common superscript differ significantly ($P<0.05$).

¹Scores of histopathological changes: 0=no significant change, 1=mild changes; 2=light changes; 3=moderate changes; 4=serious changes; 5=severe changes (NEEFF et al., 2013).

²LQ: Limit of quantification (0.5µg/kg for AFB₁, AFB₂, AFG₁ or AFG₂).

macroscopic alterations in the organs collected were noted, and no differences ($P>0.05$) were found in the scores of kidneys, heart and Bursa of Fabricius from broilers within categories. However, a higher score (1.2±0.2) was observed in liver sections of broilers from the AFs≥LOQ category, which are consistent with mild lesions including discrete steatosis, proliferation of cells of the bile ducts and periportal inflammatory infiltrate during aflatoxicosis (HUSSAIN et al., 2010). These results confirmed that even low AF levels in feed can cause negative effects in broilers, hence indicating the need for a revision of the recommended maximum value of total AFs (50µg/kg) in feed ingredients in Brazil (MINISTÉRIO DA AGRICULTURA, 1988). In agreement with our results, JONES et al. (1982) observed in the United States that low AF levels in corn, feed immediately after production and feed stored in the farm (1.2, 6.0 and 8.8µg/kg, respectively) were associated with productivity losses in apparently healthy broilers.

In conclusion, corn samples presented higher frequency of AFs (65%) and FBs (100%), at mean levels of 29.1µg/kg and 2.1mg/kg, respectively. Among the other feed ingredients, soybean meal presented the lowest frequencies for AFs (6%) and FBs (59%). Birds receiving feed containing FB levels equal or above the quantification limit (≥LOQ) of the analytical method had lower weight gain and higher mortality rates, hence indicating losses in the company's productivity. Additionally, birds fed rations with AFs≥LOQ presented higher scores of histological changes in the liver. A dilution effect was observed for AFs and FBs from corn to feed during manufacture, whilst not enough to

prevent losses in productivity. Results of this trial highlight the need for strict control of mycotoxins in corn intended for broilers.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

EK and CO conceived and designed experiments. EK performed the experiments and carried out the lab analyses. CO supervised and coordinated the animal experiments and lab analyses. CC performed statistical analyses and interpretation of experimental data. LF and RU retrieved the scientific literature and prepared the draft of the manuscript. All authors critically revised the manuscript and approved the final version.

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