INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 2, p. 137-145, 2021

RESEARCH PAPER OPEN ACCESS

Detection of aflatoxin in broiler feeds in Cebu Province, Philippines

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Key words: Aflatoxin, Detection, Feed millers, Feed retailers.

http://dx.doi.org/10.12692/ijb/18.2.137-145 Article published on February 26, 2021

Abstract

Aflatoxicosis represents one of the serious diseases of poultry, livestock and other animals attributed to *A. flavus*. Aflatoxin in feeds, when eaten by broilers, is accumulated in poultry meat and organs and considered as one of the potent food contaminants of great importance. In support to food safety and government regulations, there's a need to investigate the aflatoxin contamination in broiler feeds to determine the level of contamination in the feed millers and retailers in Southern and Northern Cebu, Philippines and to compare if there is a significant build-up of aflatoxin between feed sources from the feed mill and the feed retailing outlets. Samples were collected from randomly selected feed manufacturers and feed retailers located in Northern and Southern Cebu from January to March 2020. Samples were analyzed using the ELISA Method (Statfax ELISA Reader). The aflatoxin level for each feed sample was quantitatively determined. A result of a range of <1.4 to 20 parts per billion (ppb), indicates that the aflatoxin level is within the tolerable level. A result of more than 20 parts per billion (pbb) indicates that the aflatoxin content is above the tolerable level. Results revealed that feed samples from feed millers contain aflatoxin below the tolerable level. However, in Southern Cebu retailers, four samples consisting of starter, grower and finisher ration were found above tolerable aflatoxin level. In Northern Cebu, out of 28 samples, only 1 sample was found to contain aflatoxin above the tolerable level. Booster feed was found to contain the highest aflatoxin. The aflatoxin in broiler feeds in Cebu Province is minimal with no findings of above tolerable level (>20 ppb) at the feed millers' level. At the retailers' level, the level of contamination is 14.3% and 3.5% in Southern and Northern Cebu respectively. The aflatoxin content between feed millers and retailers in Southern Cebu is highly significantly different. There was no significant difference found between feed millers and retailers in Northern Cebu as well as the retailers in the entire Cebu Province.

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Introduction

Aflatoxin is a naturally occurring toxic metabolite produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* when exposed under extreme environmental conditions (Charoenpornsook and Karisarasai, 2006). Animal feed contamination is widespread due to the inclusion of major feed ingredients such as corn and copra meal that are susceptible to aflatoxin build-up.

Aflatoxicosis represents one of the serious diseases of poultry, livestock and other animals. The cause has been attributed to the ingestion of various feeds contaminated with *A. flavus*. The adverse effects on broilers are centered in the immune system, gastrointestinal tract and growth performance which eventually lead to economic losses. Aflatoxin poses hazards to both human and animal health. On the human health aspect, aflatoxin can be accumulated in poultry tissues and organs especially in the liver and can cause chronic and acute effects in humans consuming them (Alhousein *et al*., 2015).

Aflatoxin-contamination along the animal feed chain does not receive attention in developing countries, not knowing that it contributes to the exposure of human consumers to contaminated products (Akande *et al*., 2006). Moreover, aflatoxins cause economic impacts including increased mortality of farm animals, lowered livestock productivity, increased veterinary costs and challenges in the disposal of contaminated feeds and feed ingredients (Akande *et al*., 2006). In addition to the effects of the aflatoxins, mold-infested feeds have poor nutritional value and organoleptic properties, which affect feed intake by the animals.

When eaten by broilers, aflatoxin in feeds is accumulated in poultry meat and organs and thus considered as one of the potent food contaminants of great importance. In support of food safety and government regulations, there's a need to investigate the aflatoxin contamination in broiler feeds since no study yet has been conducted in Cebu Province, Philippines. This study supports the Food Safety Law (R.A. No. 10611) which refers to the assurance that food will not cause harm to human health and strengthening of the food safety regulatory system in the Philippines. The study determines the level of aflatoxin contamination in broiler feeds in Cebu Province, Philippines. Specifically, the study aims to determine the level of contamination of broiler feeds in the feed millers and retailers in Southern and Northern Cebu; and to compare if there is a significant build-up of aflatoxin between feed sources from the feed mill and the feed retailing outlets.

Materials and methods

Collection of broiler feed samples

Feed samples were collected from randomly selected feed manufacturers and feed retailers located in Northern and Southern Cebu, the Philippines from January to March 2020.

A written request was given to the Regional Executive Director of the Department of the Agriculture-Regional Field Office No. 7 for the permission to coordinate with the Regional Animal Feed Control Officers of the Regulatory Division of DA in the collection of 76 feed samples from selected feed millers and feed retailers in Cebu Province using random sampling following sampling protocol.

The samples were placed in a plastic bag (1.0 Kg per sample), properly labeled as to the feed type, source and location. The selection for the number of samples collected was based on Slovin's formula.

Transport of feed samples and sample preparation and storage

Collected samples were placed in bags, transported and submitted to the Regional Feed Analysis Laboratory for aflatoxin analysis. Feed samples were quartered and grounded using an analytical grinder (20 mesh). Ground samples were placed in sampling bottles and stored in the freezer ready for aflatoxin analysis.

Aflatoxin analysis of samples

Samples were analyzed for aflatoxin using the ELISA

method. Samples were extracted with 70% methanol to extract aflatoxin. Aflatoxin test kits were used wherein the aflatoxin in the extract is bound in the antibody-coated well, the basis for its quantitative determination using the Statfax ELISA Reader.

Interpretation of Aflatoxin Results of Samples

The aflatoxin level for each feed sample was quantitatively determined. A result of a range of <1.4 to 20 parts per billion (ppb), indicates that the aflatoxin level is within the tolerable level, and a result of more than 20 parts per billion (pbb) indicates that the aflatoxin content is above the tolerable level.

Statistical analysis

The data on aflatoxin content in broiler feeds were analyzed using descriptive statistics. Means and percentages of contamination were computed based on the aflatoxin results of feed samples that are within and above the tolerable level. Besides, the minimum and maximum aflatoxin detected was determined for the broiler feed types. Statistical analysis on the data on comparison of aflatoxin levels of feed sources from the feed millers versus feed samples from the retailer outlets was analyzed by T-test using Statistical Package for Social Sciences (SPSS) version 2.0.

Results and discussion

As shown in figure 1, none of the samples from feed millers were above the standard tolerable level of 20 parts per billion (ppb). Most of the samples contain aflatoxin within the range from 0 to 5 ppb comprising 60% of the 20 samples collected and the rest (40%) are within 6 to 10 ppb.

Table 1. Aflatoxin content of broiler feeds collected from feed millers by feed type, n= 20.

Feed Type	Minimum Aflatoxin Detected, ppb	Maximum Aflatoxin Detected, ppb	Mean Aflatoxin, ppb
Booster	<1.4	9.0	6.1
Starter	<1.4	7.9	4.5
Grower	5.3	9.6	7.4
Finisher	<1.4	10.3	4.4

Table 1.figure 2 reflect the minimum and maximum aflatoxin detected as well as the mean aflatoxin content of samples from feed millers by feed type.

The limit of detection of ELISA reader is less than 1.4 (<1.4) and it was the minimum level detected for most feed types except for grower feeds which are 5.3 ppb. On the other hand, the maximum level of aflatoxin detected was in finisher feed samples (10.3 ppb), followed by grower (9.6 ppb), booster (9.0 ppb) and 7.9 ppb for starter feed. The mean aflatoxin for the feed types ranges from 4.4 ppb to 7.4 ppb.

Table 2. Aflatoxin content of broiler feed samples from retailers in Southern Cebu (n= 28).

Aflatoxin Range, ppb	No. of Broiler Feed Samples	% of Total Broiler Feed Samples	% Samples	
			<20 ppb	>20 ppb
0-5	11	39.3	85.7	
6-10	3	10.7		
11-15	6	21.4		
16-20	4	14.3		
>20	4	14.3		14.3

It reveals that feed samples sourced out from feed millers have low aflatoxin levels. Most feed millers have quality control laboratories capable of analyzing aflatoxin using ELISA Reader for the monitoring of the quality of raw materials and the finished products. They are stringent on the quality of the feeds they produce especially on the level of aflatoxin Table 2, figure 3 shows the percentage of samples at a

certain aflatoxin range in ppb for broiler feed samples from retailers in Southern Cebu. Most of the samples fall within the range of 0 to 5 ppb aflatoxin (39.3%). Out of 28 samples, only 4 samples were found above the tolerable aflatoxin level (>20 ppb) equivalent to 14.3%, with 24 samples within the tolerable level (85.7%). Furthermore, table 3, figure 4 reflects by feed type the mean, minimum and maximum aflatoxin level detected of broiler feed samples sourced in Southern Cebu.

Table 3. Aflatoxin content of broiler feed samples from retailers in Southern Cebu by feed type (n=28).

Feed Type	Minimum Aflatoxin Detected, ppb	Maximum Aflatoxin Detected, ppb	Mean Aflatoxin, ppb	% Aflatoxin Contamination, >20 ppb
Booster	1.6	17.1	9.5	0
Starter	2.0	39.9	11.1	11.1
Grower	<1.4	36.3	18.2	33.3
Finisher	<1.4	33.6	9.1	14.3

Table 4. Aflatoxin content of broiler feed samples from retailers in Northern Cebu, n=28.

Aflatoxin Range, ppb	No. of Broiler Feed Samples	% of Total Broiler Feed Samples	% Samples	
			≤ 20 ppb	<20 ppb
0-5	15	53.6	96.5	
c6-10	5	17.9		
11-15	5	17.9		
16-20	2	7.1		
>20 ppb	1	3.5		3.5

Standard tolerable level of aflatoxin in broiler feeds- not more than 20 ppb.

The highest aflatoxin detected was 39.9 ppb in broiler starter feeds, followed by grower (36.3 ppb) and finisher (33.6 ppb) which are above the standard tolerable level while booster has maximum aflatoxin detected of 17.1 ppb which is within the standard tolerable level. The higher aflatoxin detected might be due to the period the feeds were stocked in the store, improper handling and storage.

Table 5. Difference in the aflatoxin level between feed millers and feed retailers in Southern Cebu.

Sample Group	No. of Samples(n)	Mean Aflatoxin, ppb	Standard Deviation	Degree of freedom (df)	T-value	p-value
Feed Millers	20	5.08	2.94	46	-2.80**	0.007
Feed Retailers Cebu South	28	12.70	12.70			

**highly significantly different at 0.01 level of significance.

Aflatoxin contamination is also influenced by the weather such as temperature and humidity. At higher moisture content, fungal growth is possible even at a lower temperature of 8 to 10°C (Mannaa and Kim, 2017). Grain heaps develop “hot spots” due to insect damage and increased physiological activity and result in moisture migration leading to fungal growth (Magan *et al.*, 2003; Peng *et al.*, 2018). Moreover, the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of agricultural commodities such as corn to fungus before they are harvested, during storage and or processing periods. Relative humidity, temperature and kernel moisture are factors affecting the physiological processes of fungi.

Table 6. Difference in the aflatoxin level between the broiler feeds from feed millers and Northern Cebu retailers.

Sample Group	No. of Samples (n)	Mean Aflatoxin, ppb	Standard Deviation	Degree of freedom (df)	T-value	p-value
Feed Millers	20	5.08	2.94	46	-1.34ns	0.187
Feed Retailers Cebu North	28	7.60	8.04			

ns- not significantly different (the means).

The aflatoxin level of broiler feed samples sourced out from feed retailers in Northern Cebu is shown in Table 4, figure 5. Out of 28 samples, only 1 sample was found to contain aflatoxin above the tolerable level (>20 ppb) equivalent to 3.5% of the total samples, the rest are below 20 ppb aflatoxin. Besides, Table 5, Figure 6 show the mean, minimum and maximum aflatoxin detected in Northern Cebu by feed type. It can be seen that among four feed types, the booster was found to contain the highest aflatoxin detected (39.5 ppb), followed by grower (18.1 ppb), starter (16.9 ppb) and the least in finisher feed (6.6 ppb). Figure 6 shows the level of aflatoxin contamination of broiler feeds in Cebu Province.

Table 7. Difference in the aflatoxin level between the broiler feeds from feed retailers in Southern and Northern Cebu.

Sample Group	No. of Samples (n)	Mean Aflatoxin, ppb	Standard Deviation	Degree of freedom (df)	T-value	p-value
Feed Retailers Cebu South	28	12.70	11.90	54	1.88ns	0.187
Feed Retailers Cebu North	28	7.60	8.04			

ns- not significantly different (the means).

Results show that there is minimal aflatoxin occurrence of samples sourced out from feed millers (mean of 5.08 ppb) with none above the standard tolerable level. As the feeds are distributed in the retailers, the aflatoxin content increases to 12.70 ppb and 7.60 ppb in Southern and Northern Cebu respectively. Southern Cebu has 14.3 % aflatoxin contamination above the tolerable level (>20 ppb), higher than in Northern Cebu which is only 3.5%.

The result of the survey by Korrapati Kotinagu, *et al.* (2015) on the factors predisposing mycotoxins in poultry feeds was centered into the general feed storage and feed purchasing.

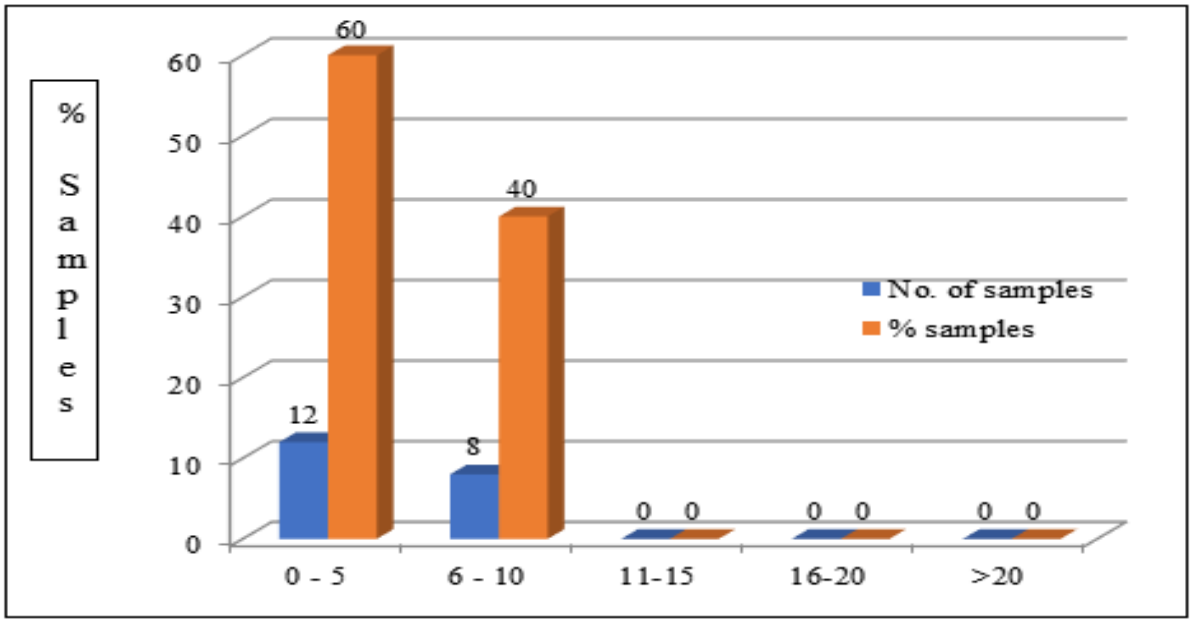

Fig. 1. Aflatoxin content of broiler feed samples collected from feed millers.

In their study, all the 44 respondents practiced stockpiling of the feeds and feed ingredients especially maize bran (81.8%) in preparation for seasons of scarcity. The majority (88.6%) of the respondents reported having encountered molds in their feeds and ingredients during storage while 72.7% did not practice the first-in-first-out good store management practice.

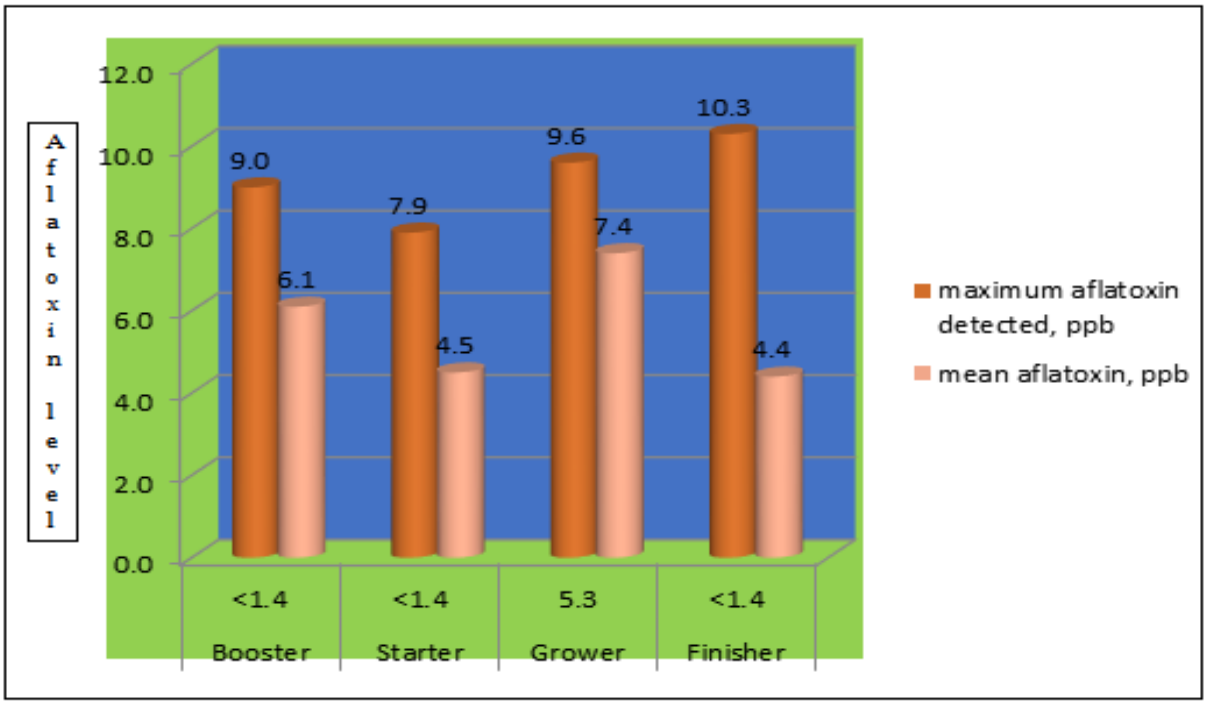

Fig. 2. Aflatoxin conten of broiler feeds collected from feed millers.

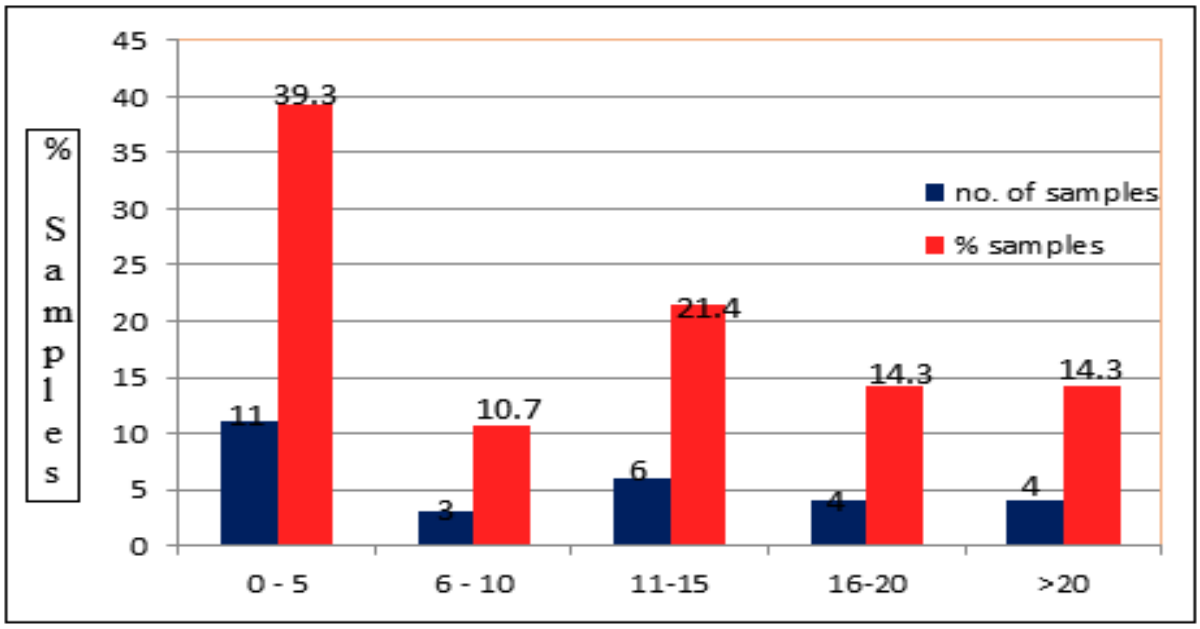

Fig. 3. Aflatoxin content of broiler feed samples from retailers in Southern Cebu.

The greatest bulk of animal and poultry feeds is composed of raw materials that are mainly cereal-based, and these are often prone to contamination by mycotoxin-producing fungi. Food and Agricultural Organisation (FAO) estimated up to 25% of the world's food crops and a big proportion of the world's animal feedstuffs being contaminated by mycotoxins (Streit *et al.*, 2013) Moreover, in a review by Bankole *et al.* (2006), it was pointed out that maize and groundnuts are the most contaminated crops with *Aspergillus* spp. in Sub Saharan Africa.Stockpiling and other poor bulk handling practices of feeds, including prolonged time in storage, predisposes to contamination of feedstuffs with aflatoxin-producing

fungi (Cheat *et al.*, 2016; Makau *et al.*, 2016). Moreover, stockpiling and direct storage of feeds/ingredients on the floor increases the dampness and moisture content, while the pressure exerted by feeds upon each other leads to reduced feed particle sizes hence creating conditions favorable for mold growth (Munthali *et al.*, 2016). There is a significant difference in the build-up of aflatoxin for broiler feed samples sourced from feed millers and feed retailers, T-test was employed and as shown in Table 5, the aflatoxin level of broiler feeds from feed millers and Southern Cebu retailers are highly significantly different ($P<0.01$). On the other hand, there is no significant difference in the aflatoxin content of broiler feeds from feed millers and retailers in Northern Cebu as shown in Table 6.

Furthermore, the aflatoxin content of broiler feeds from retailers in Southern and Northern Cebu are not significantly different (Table 7).

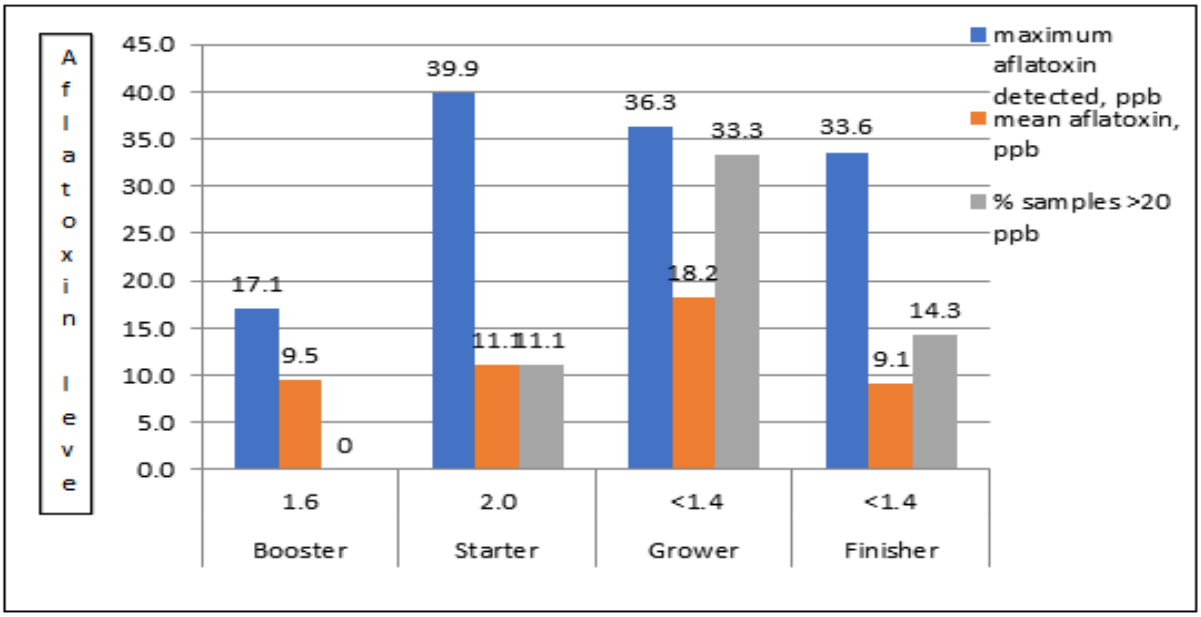

Fig. 4. Aflatoxin content of broiler feed samples from retailers in Southern Cebu by feed type.

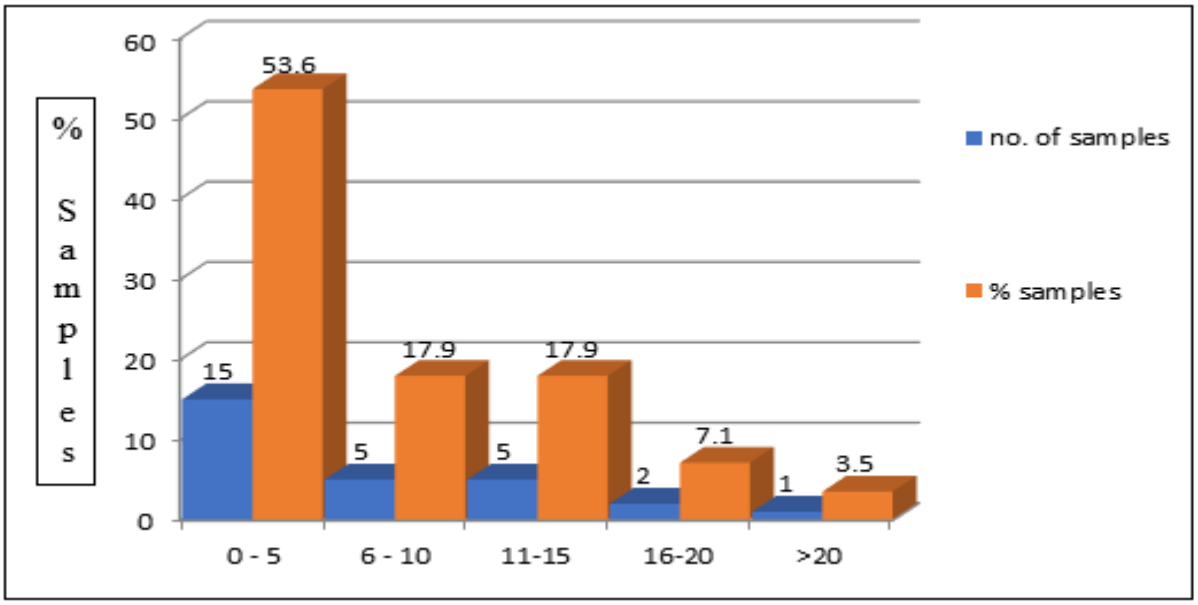

Fig. 5. Aflatoxin content of broiler feed samples from retailers in Northern Cebu.

The occurrence of aflatoxin in broiler feeds in Cebu Province is minimal. Most samples were below the regulatory limit and quality was maintained at the feed miller's level. At the retailers' level, the contamination is 14.3% and 3.5% in Southern and Northern Cebu respectively.

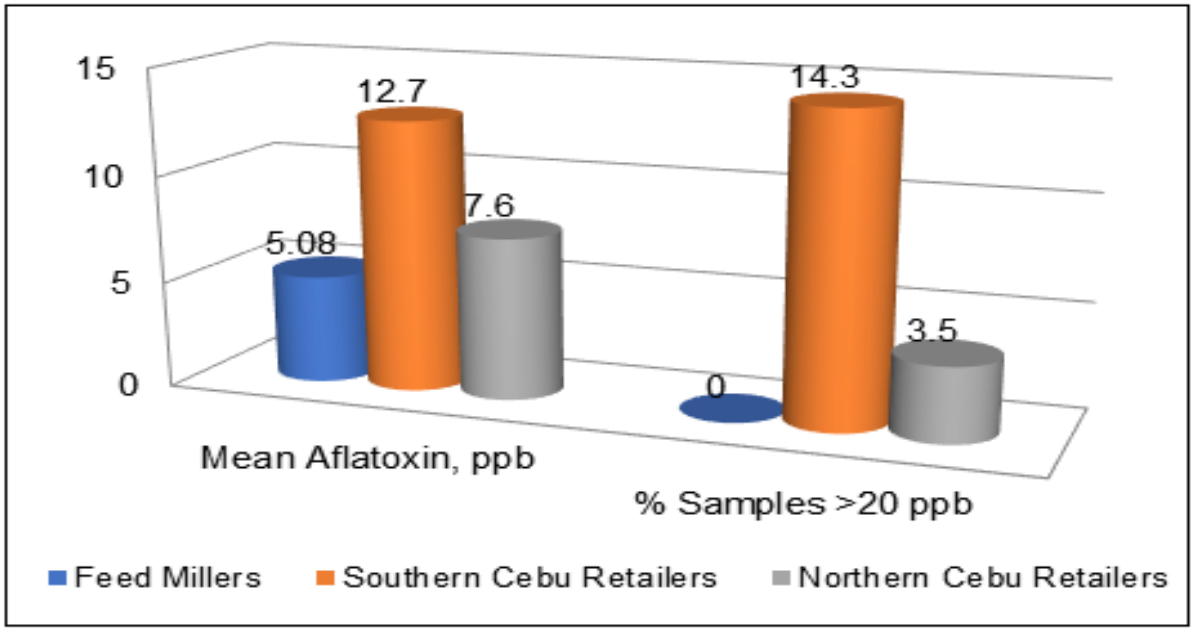

Fig. 6. Level of aflatoxin contamination of broiler feeds in Cebu Province.

The aflatoxin content between feed millers and retailers in Southern Cebu is highly significantly different but no significant difference was found between feed millers and retailers in Northern Cebu as well as the retailers in the entire Cebu Province. The lower levels of aflatoxins in poultry feed types from large scale processors compared to those from small scale processors is influenced by the improved processing and storage facilities as well as the short holding duration among the former category.

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