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Research Article
Aflatoxin and Ochratoxin A Contamination in Corn Grains and Sago (*Putak* Meal) from Different Sources for Poultry in West Timor, Indonesia

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Abstract

Objectives: The main objective of the present study was to evaluate Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A contamination in corn grains and sago (*putak* meal) in some areas of West Timor, Indonesia. However, because information regarding antinutritional factors in sago is limited, our other objective was to evaluate the content of neutral detergent fiber (NDF), phytic acid and tannins in sago. **Materials and Methods:** A total of 30 corn samples and whole *putak* samples from four different sources were collected from traditional markets, farmers and feed mills in Kupang, South Central Timor (SCT), Belu and Malaka regencies in West Timor, Indonesia. Aflatoxins and Ochratoxins were measured using Thin Layer Chromatography (TLC). **Results:** The results showed that the percentage of sago (*putak* meal) contaminated with Aflatoxins (B1, B2, B3 and B4) was 0% but that contamination with Ochratoxins was 50% and the amount was 20.385 μg kg^{-1}. Corn grains from farmers and traditional markets in Kupang regency were not found to be contaminated with Aflatoxins (B1, B2, B3 and B4) or Ochratoxin A (OTA); only one corn sample from feed mills was contaminated with Aflatoxin B1 (12.18 μg kg^{-1}). In SCT regency, among the 8 samples analyzed (from traditional markets and farmers), only one sample from a farmer was contaminated with Aflatoxin B1 (24.35 μg kg^{-1}). Corn samples obtained from traditional markets and farmers in the Belu and Malaka regencies were contaminated with Aflatoxin B1 (AFB1) at a concentration of 65.50 and 24.36 μg kg^{-1}, respectively. **Conclusion:** In conclusion, all Sago (*putak* meal) was found to be free from Aflatoxins but 50% was contaminated by Ochratoxin A. The majority of corn samples obtained from traditional markets and farmers in West Timor were free from Aflatoxin and Ochratoxin A contamination. The contamination of Ochratoxin A in sago and Aflatoxin B1 in corn samples taken from traditional markets, farmers and feed mills in West Timor was probably a result of improper storage and natural contamination. *Huma* and metal drums could maintain the quality of the corn samples.

Key words: Aflatoxins, corn grains, ochratoxin A, sago (putak meal), West Timor

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Corn is the main energy source for the poultry diet formulation in Indonesia. Corn grains used in the poultry industry originate from some provinces in Indonesia and are also imported from overseas (i.e. the USA and Argentina). The Data Center and Information System of the Indonesian Ministry of Agriculture reported that corn production in Indonesia during the period 2011-2015 was primarily from ten provinces with a contribution of 89.47% of the national total production[1]. Furthermore, it was reported that the highest production of corn was from East Java (30.93%), followed by that of Central Java (15.89%) and Lampung (9.3%). East Nusa Tenggara including West Timor was in the ninth position with a total contribution of 3.4%.

The price of corn grains at the producer level varies depending on the moisture level. The lower the moisture content of corn the higher the price and vice versa. A lower price for corn is associated with high moisture (25-30%) and Aflatoxin content[1]. Currently, the price of corn grains at the farmer level in Indonesia is USD 0.23 kg^{-1}, while the corn price in traditional markets in West Timor, Indonesia is USD 0.47 kg^{-1}.

In West Timor, one of the alternative energy sources used for diet formulation is sago (*putak* meal), which is obtained from the pith of the mature *gebang's* trunk (*Corypha utan*)[2,3]. According to Nalle *et al.*[2], sago (*putak* meal) contains 11.62 MJ kg^{-1} apparent metabolisable energy (AME) and it can be included at up to 200 g kg^{-1} in broiler diet. The gebang tree can be abundantly found not only in Indonesia but also in other countries such as northern Australia, Malaysia, New Guinea, India and South East Asia[3]. According to Eagleton[3], the height of the gebang tree during its final age stage is 25-30 m, with the diameter of the trunk reaching 1-2.5 m. *Gebang* trees in West Timor are very abundant. The Directorate General of Animal Husbandry[4] reported that approximately 4.876 ha of Timor Island Pasture grow *gebang* trees. Sago (*putak* meal) is obtained from the *gebang* tree through a series of processes, starting with removal of the outer skin of the tree stem and then cutting the stem into several pieces. Local people term the stem of the *gebang* tree after removal the outer skin *putak* (sago). Based on the present study, the weight of each piece of *putak* sold ranges from 11.42-24.70 kg. After grinding, drying and sieving, each piece of *putak* produces sago (*putak* meal) at approximately 43.8% of the total weight and fiber (13.05% of the total weight). Generally, traders sell the whole *putak* with high moisture content, not as ground *putak* (sago). Thus, the high moisture content of the whole *putak* is good media for the growth of fungi which can lead to mycotoxin production such as Aflatoxins and Ochratoxins.

Aflatoxins are the most common mycotoxin, having carcinogenic and teratogenic properties and produced by *Aspergillus flavus* and *Aspergillus parasiticus*[5]. *Aspergillus flavus* produces the B1 and B2 types, whereas *Aspergillus parasiticus* produces B1, B2, G1 and G2 types. Aflatoxins can have negative impacts on animal and human growth. The toxicity of Aflatoxins depends on their level in feed and it differs for each animal species. Chickens are the most susceptible to acute aflatoxicosis compared to other poultry[6]. Animal farmers and feed formulators who produce diets using mycotoxin-contaminated corn may face problems related to animal health, production and reproduction performance. Aflatoxin B1 residue in edible animal products is responsible for liver cancer in humans.

Another type of fungal toxin that is also dangerous to animals and humans is the family of Ochratoxins. Ochratoxins are produced mostly by *Aspergillus ochraceus* and *Penicillium verrucosum*. Both *Aspergillus ochraceus* and *Penicillium verrucosum* produce type A, B and C Ochratoxins. *Aspergillus ochraceus* might be responsible for Ochratoxin A contamination in hot tropical regions, whereas *Penicillium verrucosum* is the main ochratoxigenic in cool-temperate climates[7]. The negative effects of Ochratoxin are a reduction in body weight gain and a change in the intestinal epithelial cell of animals. Ochratoxin A has been found to be nephrotoxic, neurotoxic, immune-toxic, genotoxic and carcinogenic in humans[8]. Based on the aforementioned discussion, the main objective of the present study was to evaluate the contamination of Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A in corn grains and sago (*putak* meal) in some areas in West Timor, Indonesia. In addition, because the published data regarding antinutritional factors in sago is limited, another objective was to evaluate the content of neutral detergent fiber (NDF), phytic acid and tannins in sago.

MATERIALS AND METHODS

Ingredients: A total of 30 samples of corn grains were randomly collected from traditional markets, feed mills and farmers in four regencies in West Timor. *Putak* (*gebang* tree stem that has been removed from its outer skin) was obtained from a traditional market in the Kupang area.

Sampling and sample preparation: Sampling and sample preparation procedures for the corn sample were as follows:

- Corn grains were sampled from traditional markets, feed mills and farmers using the percentage method (10%)[9]

- Samples from each trader, farmer and feed mill were then mixed to produce an incremental sample
- The incremental samples were then reduced using the Conning-Quartering Classical method[10] to produce an aggregate/reduced sample. The aggregate samples were then ground using a disk mill with a screen size of 3 mm
- The aggregate samples were then reduced using a Cone Sampler Divider to obtain laboratory samples. At the end of the process, there were 8 bottle containers filled with ground samples
- The laboratory samples were then ground using a sample mill with a screen size of 0.5 mm, packed and sent to the laboratory for moisture content, Aflatoxin (B1, B2, G1 and G2) and Ochratoxin A analysis

The sampling and sample preparation procedures for sago (*putak* meal) were as follows:

- *Putak* (*gebang* tree stem that has been removed from their outer skin) was sampled from traditional markets using the percentage method (10%)[9]
- *Putak* samples from each trader were then chopped, ground using a hammer mill (3 mm screen size), sun-dried, sieved and mixed to produce increment samples (Fig. 1)
- The incremental samples were then reduced using the Conning-Quartering Classical method[10] to produce aggregate/reduced samples
- The aggregate samples were then reduced using a Cone Sampler Divider to obtain laboratory samples
- The laboratory samples were then ground using a sample mill (0.5 mm screen size), packed and sent to the laboratory for proximate, gross energy, NDF, tannin, phytic acid, Aflatoxin (B1, B2, G1, G2) and Ochratoxin A analysis

Chemical analysis: Moisture content (AOAC 930.15, AOAC 925.10 AOAC)[9], crude protein content (AOAC 2001.11)[9], crude lipid (AOAC 2003.06)[9] and NDF (Van Soest Method) AOAC 942.5[9], were analyzed. Aflatoxin (AOAC Official Methods 993.17)[11] was analyzed using Thin Layer Chromatography. Aflatoxin was extracted from the ground sample using methanol-water. The filtrate was then diluted using a NaCl solution and defatted with n-hexane. Aflatoxin was partitioned into chloroform which was then removed via evaporation. Aflatoxin was purified via chromatography on a silica gel column and quantified using thin layer chromatography (TLC) on silica gel 60 via visual estimation. Ochratoxin A was extracted from the ground sample using acetonitrile. The filtrate was defatted with n-hexane. Ochratoxin A analysis was conducted using a previously described method[12]. Ochratoxin was partitioned into dichloromethane which was then removed via evaporation. Aflatoxin was purified via chromatography on a silica gel column and quantified using thin layer chromatography (TLC) on silica gel 60 via visual estimation. The spectrophotometry method was used to determine the phytic acid and tannin concentrations.

RESULTS

Proximate and antinutritional factors and aflatoxin and Ochratoxin A contamination in sago (*putak* meal): As listed in Table 1, the proximate contents of sago (*putak* meal) consisted of 23.87-28.49 g kg^{-1} DM for crude protein, 13.17-39.09 g kg^{-1} DM for crude lipids, 43.81-59.30 g kg^{-1} DM for crude fiber and 23.10-41.08 g kg^{-1} DM for ash. The neutral detergent fiber (NDF) content of sago ranged from 188.7-234.5 g kg^{-1} DM, while tannin and phytic acid contents in the sago were between 1.25-2.27 g kg^{-1} DM and

Fig. 1: Sago (*putak* meal) sample preparation

Table 1: Proximate, NDF, tannin and phytic acid contents of sago (*putak* meal) in West Timor, Indonesia

		g kg^{-1} DM							
Sample	Dry matter (g kg^{-1})	Crude protein	Crude lipid	Crude fiber	Ash	NDF*	Tannin**	Phytic acid**	Gross energy MJ kg^{-1} DM
Sample 1	887.90	28.490	13.170	43.810	25.110	234.500	1.130	29.620	17.670
Sample 2	882.90	27.520	21.180	55.500	38.390	219.600	2.270	33.750	16.390
Sample 3	883.50	24.900	17.200	40.290	41.080	188.700	1.250	30.440	17.880
Sample 4	900.40	23.870	39.090	59.300	23.100	215.600	2.220	28.650	17.960
Average	888.70	26.200	22.660	49.720	31.920	214.600	1.710	30.610	17.480
SEM	4.06	1.084	5.716	4.556	4.564	9.543	0.306	1.107	0.367

Each value is the average of 2 replicates *Test method: Van Soest; **Test method: Spectrophotometry

Table 2: Aflatoxin and Ochratoxin A contents of sago (*putak* meal) in West Timor, Indonesia

			Aflatoxins* (µg kg^{-1} DM)				
Sample	Duration of storage	Moisture content (%)	B1	B2	G1	G2	Ochratoxin A**
Sample 1	<3 days	11.07	0.00	0.00	0.00	0.00	20.38
Sample 2	<3 days	11.21	0.00	0.00	0.00	0.00	20.39
Sample 3	<3 days	11.71	0.00	0.00	0.00	0.00	0.00
Sample 4	<3 days	9.96	0.00	0.00	0.00	0.00	0.00

Each value is the average of 2 replicates *Test method: TLC (thin layer chromatography) with a detection limit for B1: 2.02 µg kg^{-1}, B2: 3.50 µg kg^{-1}, G1: 0.54 µg kg^{-1} and G2: 1.00 µg kg^{-1}, **Test method: TLC (thin layer chromatography) with a detection limit for Ochratoxin A: 0.48 µg kg^{-1}

Table 3: Percentage of corn samples contaminated by Aflatoxins and Ochratoxin A in some areas of West Timor

			Aflatoxins (%)				
Location	Sample source	N	B1	B2	G1	G2	Ochratoxin A
Kupang	Farmers	2	0.0	0.0	0.0	0.0	0.0
	Traditional markets	7	0.0	0.0	0.0	0.0	0.0
	Feed mills	4	25.0	0.0	0.0	0.0	0.0
					0.0	0.0	0.0
SCT**	Farmers	3	33.3	0.0	0.0	0.0	0.0
	Traditional markets	5	0.0	0.0	0.0	0.0	0.0
Belu/Malaka	Farmers	6	16.7	0.0	0.0	0.0	0.0
	Traditional markets	3	33.3	0.0	0.0	0.0	0.0

Each value is the average of 2 replicates*, **South Central Timor

28.65-33.75 g kg^{-1} DM, respectively. Sago was rich in energy content, 16.39-17.96 MJ kg^{-1} DM. Table 2 describes the Aflatoxin and Ochratoxin A contents in the four sago (*putak* meal) samples in the Kupang area, West Timor, Indonesia. All samples were determined to be free from Aflatoxins (B1, B2, G1 and G2) but 50% of the samples were infected by Ochratoxin A (OTA). The level of Ochratoxin A contents detected in these samples were 20.38 µg kg^{-1} DM and 20.39 µg kg^{-1} DM, respectively.

Aflatoxins and Ochratoxin A contamination in corn samples: Table 3 lists the percentage of corn grain samples contaminated by each type of Aflatoxin and Ochratoxin A in some areas in West Timor, Indonesia. The results showed that none of the corn samples obtained from traditional markets in the Kupang and SCT regencies were contaminated by AFB1, AFB2, AFG1, AFG2 and Ochratoxin A. One-third of the corn samples (33%) obtained from farmers in SCT contained AFB1. In the Belu/Malaka regency, only 16.7% of the corn samples from the farmers were contaminated by AFB1. The percentage of corn samples collected from the feed mills contaminated by AFB1 was only 25%.

Table 4-6 list the content of Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A in 13 corn grain samples obtained from traditional markets, farmers and feed mills in the Kupang, SCT and Belu/Malaka regencies in West Timor, Indonesia. In the Kupang regency, the results showed that seven corn grain samples taken from traditional markets and two corn samples obtained from farmers were not contaminated (0.00 µg kg^{-1}) by any of the types of Aflatoxins and Ochratoxin A. There was only one corn sample (25%) from a feed mill that was observed to be contaminated by Aflatoxin B1 and it was at a concentration of 12.18 µg kg^{-1}.

All corn samples from the traditional markets in the SCT regency were not contaminated by Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A. However, one sample from a farmer was contaminated by Aflatoxin B1 (24.35 µg kg^{-1}) (Table 4).

Table 4: Aflatoxin and Ochratoxin A contents in corn grains from different sources in the Kupang area, West Timor, Indonesia

			Aflatoxins* (μg kg^{-1} DM)				
Sample source	Sample storage duration	Moisture content (%)	B1	B2	G1	G2	Ochratoxin A**
Traditional markets							
Sample 1	<1 year	11.25	0.00	0.00	0.00	0.00	0.00
Sample 2	<1 year	11.90	0.00	0.00	0.00	0.00	0.00
Sample 3	<1 year	11.38	0.00	0.00	0.00	0.00	0.00
Sample 4	<1 year	10.67	0.00	0.00	0.00	0.00	0.00
Sample 5	<1 year	11.54	0.00	0.00	0.00	0.00	0.00
Sample 6	<1 year	13.32	0.00	0.00	0.00	0.00	0.00
Sample 7	<1 year	13.32	0.00	0.00	0.00	0.00	0.00
Farmers							
Sample 1	<1 year	12.23	0.00	0.00	0.00	0.00	0.00
Sample 2	<1 year	11.07	0.00	0.00	0.00	0.00	0.00
Feed mills							
Sample 1	1 year	11,07	0.00	0.00	0.00	0.00	0.00
Sample 2	1 year	10.68	0.00	0.00	0.00	0.00	0.00
Sample 3	2 months	10.26	0.00	0.00	0.00	0.00	0.00
Sample 4	2 year	12.38	12.18	0.00	0.00	0.00	0.00

Each value is the average of 2 replicates *Test method: TLC (Thin layer chromatography) with a detection limit for B1: 2.02 μg kg^{-1}, B2: 3.50 μg kg^{-1}, G1: 0.54 μg kg^{-1} and G2: 1.00 μg kg^{-1}, **Test method: TLC (Thin layer chromatography) with a detection limit for Ochratoxin A: 0.48 μg kg^{-1}

Table 5: Aflatoxin and Ochratoxin A contents in corn grains from different sources in South Central Timor (SCT) regency, West Timor, Indonesia

			Aflatoxins* (μg kg^{-1} DM)				
Sample source	Sample storage duration	Moisture content (%)	B1	B2	G1	G2	Ochratoxin A**
Traditional markets							
Sample 1	<1 year	11.70	0.00	0.00	0.00	0.00	0.00
Sample 2	<1 year	10.65	0.00	0.00	0.00	0.00	0.00
Sample 3	<1 year	9.80	0.00	0.00	0.00	0.00	0.00
Sample 4	<1 year	12.05	0.00	0.00	0.00	0.00	0.00
Sample 5	<1 year	11.95	0.00	0.00	0.00	0.00	0.00
Farmers							
Sample 1	<1 year	12.05	0.00	0.00	0.00	0.00	0.00
Sample 2	<1 year	12.25	24.35	0.00	0.00	0.00	0.00
Sample 3	<1 year	11.65	0.00	0.00	0.00	0.00	0.00

Each value is the average of 2 replicates *Test method: TLC (thin layer chromatography) with a detection limit for B1: 2.02 μg kg^{-1}, B2: 3.50 μg kg^{-1}, G1: 0.54 μg kg^{-1} and G2: 1.00 μg kg^{-1}, **Test method: TLC (thin layer chromatography) with a detection limit for Ochratoxin A: 0.48 μg kg^{-1}

Table 6: Aflatoxin and Ochratoxin A contents in corn grains from different sources in Belu and Malaka regencies, West Timor, Indonesia

			Aflatoxins* (μg kg^{-1} DM)				
Sample source	Sample storage duration	Moisture content (%)	B1	B2	G1	G2	Ochratoxin A**
Traditional markets							
Sample 1	1 year	10.20	0.00	0.00	0.00	0.00	0.00
Sample 2	1 year	10.65	0.00	0.00	0.00	0.00	0.00
Sample 3	1 year	12.65	65.50	0.00	0.00	0.00	0.00
Farmers							
Sample 1	<4 months	10.55	0.00	0.00	0.00	0.00	0.00
Sample 2	<4 months	11.35	0.00	0.00	0.00	0.00	0.00
Sample 3	<4 months	12.20	24.36	0.00	0.00	0.00	0.00
Sample 4	<4 months	10.85	0.00	0.00	0.00	0.00	0.00
Sample 5	4 years	11.30	0.00	0.00	0.00	0.00	0.00
Sample 6	<4 months	11.45	0.00	0.00	0.00	0.00	0.00

Each value is the average of 2 replicates *Test method: TLC (Thin layer chromatography) with a detection limit for B1: 2.02 μg kg^{-1}, B2: 3.50 μg kg^{-1}, G1: 0.54 μg kg^{-1} and G2: 1.00 μg kg^{-1}; **Test method: TLC (Thin layer chromatography) with a detection limit for Ochratoxin A: 0.48 μg kg^{-1}

As seen from Table 5, among the 9 samples collected, there were only two samples infected by Aflatoxin B1, at a concentration of 65.50 μg kg^{-1} for a corn sample obtained from the traditional market and 24.36 μg kg^{-1} for a corn sample from a farmer. No Ochratoxin A contamination was found in corn grains obtained from farmers and traditional markets in the Belu/Malaka regencies, West Timor.

Fig. 2: *Huma* and metal drum for corn storage

In general, as shown in Table 3-5, among 30 corn samples analyzed, only four samples (10.3%) were contaminated by Aflatoxin B1 at a concentration between 12.18-65.50 μg kg^{-1}. Among 15 corn samples collected from traditional markets, only one sample (6.6%) was contaminated by AFB1. Notably, approximately 90% of corn grain samples obtained from farmers in all regencies in West Timor were free from Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A. This was probably a result of a number of factors such as the moisture content of the corn when harvested and the storage method applied by farmers in West Timor. The moisture content of the corn grain samples obtained from the farmers was <14%. The farmers in West Timor stored their corn in a location termed a *huma* (a small house composed of materials from the *gebang* tree, Fig. 2). Inside the *huma*, some farmers placed the corn above a wood rack attached to the roof and smoked it using firewood under the rack. The heat and smoke from the fire reduced the moisture content of the corn and prevented weevils from breaking the corn. Other farmers stored their corn grain inside metal drums (Fig. 2). Corn grain stored in metal drums was not easily infested by weevils and fungi. Although, one sample of corn grain (sample 5, Table 5) that had been stored for four years in a metal drum was free from Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A, compared to corn grains (Sample 4) collected from a feed mill (stored in a plastic sack for 2 years) that were already contaminated by Aflatoxin B1 (AFB1), this result indicated that a metal drum could maintain the quality of corn grains because the drum container prevented moisture from entering the drums. In contrast, a plastic sack is porous and can allow moisture to freely, creating a good environment for fleas and fungi to grow.

The range of AFB1 detected in corn samples (from 12.18-65.50 μg kg^{-1}) was still within the good category based on the Indonesian National Standard for Aflatoxin contamination in corn.

DISCUSSIONS

Aflatoxin and ochratoxin contamination in sago (putak meal*).* Notably, Table 1 shows that the crude protein (CP) (26.20 g kg^{-1} DM) and NDF content (214.6 g kg^{-1} DM) in the present study was lower than that reported by Nalle *et al.*[2], (36.6 g kg^{-1} DM and 267.5 g kg^{-1} DM), respectively. The discrepancies were probably a result of the different age of *putak* used and the soil condition. In addition, grinding and screening methods were different. In the present study, *putak* was ground using a commercial hammer mill and then manually screened, while Nalle *et al.*,[2] used a special homemade *putak* grinder attached to screener in four different screen sizes.

In addition to neutral detergent fiber (NDF), it was discovered that sago also contains phytic acid and tannin. The phytic acid content of sago was quite high (30.61 g kg^{-1} DM) compared to that of rice bran (50.5-84.8 g kg^{-1} DM)[13]. The tannin content in the sago ranged from 1.13-2.27 g kg^{-1} DM.

The contamination of OTA in the sago samples was probably a result of two main factors, i.e., inappropriate storage and natural contamination before harvesting. Raw and wet *putak* were found to contain approximately 57% moisture content and they were kept by the trader on the street floor without a pallet for approximately three days before being sold. This was a good media for fungal growth. Addressing natural contamination, it was suspected that the *gebang* tree had been contaminated by Ochratoxin A before being cut by the farmer.

The amount of Ochratoxin A (OTA) found in the sago (*putak* meal) was quite high, at an average of 20.385 μg kg^{-1} DM. Regulation regarding the maximum Ochratoxin level in feed ingredients has not been implemented as of yet but most agree that concentrations between 10 and 20 ppb for feed ingredients can result in health problems and potential economic losses[14]. Published data have showed that an OTA concentration of 2 mg kg^{-1} caused an enlarged kidney and liver in growing broilers. In addition, the color of the chicken liver becomes pale and friable[15]. Chicks fed a diet containing a high concentration of OTA (400 ppb and 800 ppb) had lower body weight, reduced feed intake, reduced feed per gain and decreased blood cell count compared to those of the control diet (no Ochratoxin)[16].

Ochratoxin A has been found to be poisonous to the nervous system, causing developmental malformation and suppression of the immune response[10]. In a review by Bui-Klimke and Wu[17], it was noted that Ochratoxin has been identified as a renal carcinogen in a variety of animal species. In addition, the author stated that the International Agency for Research on Cancer (IARC) categorized OTA as a group 2B possible human cancer-causing agent, based on their experiments using laboratory animals. Indresh and Umakantha[18] reported that birds fed a diet containing Ochratoxin A had poor performance, low lymphoid organ weight, low serum total protein, albumin and antibody titers for IBD and ND.

Aflatoxin and ochratoxin contamination in corn grains. The maximum concentration of Aflatoxins in corn is 100 ppb for Grade I and 150 ppb for Grade II[19].Meanwhile, according to the National Grain and Feed Association[20], the safety level of corn Aflatoxin for young and mature poultry is 20 and 100 ppb, respectively. The result of the present study indicates that three out of four corn samples contaminated with AFB1 were not suitable for immature poultry but could still be used for mature poultry.

The majority of corn samples contaminated with AFB1 had a moisture level >12.0%. This moisture level is actually within the range of the Indonesian National Standard requirement for corn grains. However, the contamination was probably a result of the improper storage. In traditional markets, for example, traders place corn inside a wooden box without a lid. This could encourage fleas to damage the corn grains. The broken corn is wet and becomes a good media for fungi to grow and produce toxins.

Based on the present result, it is quite crucial for the government to provide a standard regulation for the maximum level of Ochratoxin in sago (*putak* meal) used in poultry and other animal diet formulation. Regarding the high level of Aflatoxin B1 discovered in the corn grain samples from traditional markets and feed mills, it is important for corn traders and quality control staff in feed mills to consider proper storage. The possible use of a mold inhibitor is urgently suggested for feed suppliers. The application of a hazard analysis and a critical control point (HACCP) for mycotoxin in a feed mill should be considered to maintain quality assurance of feed products.

CONCLUSION

All Sago (*putak* meal) was found to be free from Aflatoxins but 50% of the samples were contaminated by Ochratoxin A. The majority of corn samples obtained from traditional markets and farmers in West Timor were free from Aflatoxin and Ochratoxin A contamination. The contamination of Ochratoxin in sago (*putak* meal) and Aflatoxin B1 in corn sample taken from the traditional markets, farmers and feed mills in West Timor was probably a consequence of inadequate storage and natural contamination. *Huma* and metal drums could maintain the quality of corn samples.

SIGNIFICANCE STATEMENT

This study determined the level of contamination from Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A in corn grain and sago (*putak* meal) that can be used in the poultry feed industry and by the government in implementing a regulation regarding the maximum standard of mycotoxin in feed. This study will help researchers to determine the critical area of mycotoxin contamination in poultry feed ingredients in West Timor, which many researchers have not able to investigate. Thus, a new theory regarding certain mycotoxins (Aflatoxins and Ochratoxin A) and possibly other mycotoxins, in feed/feed ingredients and a strategy to prevent their introduction and eliminate them may be developed.

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