Aflatoxin analysis of dairy feeds and milk in the Greater Addis Ababa milk shed, Ethiopia

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INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

CGIAR RESEARCH PROGRAM ON Agriculture for Nutrition and Health
Led by IFPRI

LIVES
Livestock and Irrigation Value chains for Ethiopian Smallholders

Aflatoxins

- Toxic secondary metabolites produced by *Aspergillus* fungi
- Contaminate a variety of foods such as corn, oil seed and animal feed
- One of the most toxic forms of aflatoxin (AFB1) is converted to AFM1 and excreted in milk by lactating animals that consume contaminated feed
- Highly carcinogenic, cause liver cancer, stunting and immunosuppression

Aspergillus flavus (Maize breeding program at Texas A&M University)

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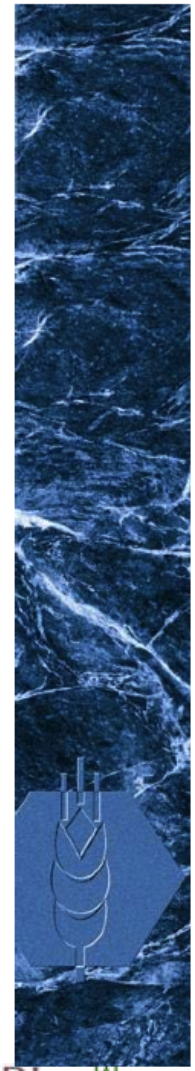
Aflatoxin Regulatory Guidance



FDA Mycotoxin Regulatory Guidance

A Guide for Grain Elevators, Feed Manufacturers, Grain Processors and Exporters

National Grain and Feed Association
1250 Eye St., N.W., Suite 1003, Washington, D.C., 20005-3922
Phone: (202) 289-0873 Fax: (202) 289-5388
Web Site: www.ngfa.org

August 2011

FDA's Action Levels for Aflatoxin

FDA has established the following action levels for aflatoxins present in human food, animal feed and animal feed ingredients as indicated in Chart 1.

Chart 1: FDA Action Levels for Aflatoxin in Human Food, Animal Feed and Animal Feed Ingredients

Intended Use	Grain, Grain By-Product, Feed or other Products	Aflatoxin Level [parts per billion (p.p.b.)]
Human consumption	Milk	0.5 p.p.b. (aflatoxin M1)
Human consumption	Foods, peanuts and peanut products, brazil and pistachio nuts	20 p.p.b.
Immature animals	Corn, peanut products, and other animal feeds and ingredients, excluding cottonseed meal	20 p.p.b.
Dairy animals, animals not listed above, or unknown use	Corn, peanut products, cottonseed, and other animal feeds and ingredients	20 p.p.b.
Breeding cattle, breeding swine and mature poultry	Corn and peanut products	100 p.p.b.
Finishing swine 100 pounds or greater in weight	Corn and peanut products	200 p.p.b.
Finishing (i.e., feedlot) beef cattle	Corn and peanut products	300 p.p.b.
Beef, cattle, swine or poultry, regardless of age or breeding status	Cottonseed meal	300 p.p.b.

EU

0.05 p.p.b.

4 p.p.b.

The following additional policies and legal provisions concerning aflatoxin also are important:

- **FDA Blending Policy:** Importantly, with respect to aflatoxin, FDA currently generally does **not** permit corn containing aflatoxin to be blended with uncontaminated corn to reduce the aflatoxin content of the resulting mixture to levels acceptable for use as human food or animal feed. However, on occasion FDA has relaxed its "no-blending" policy in

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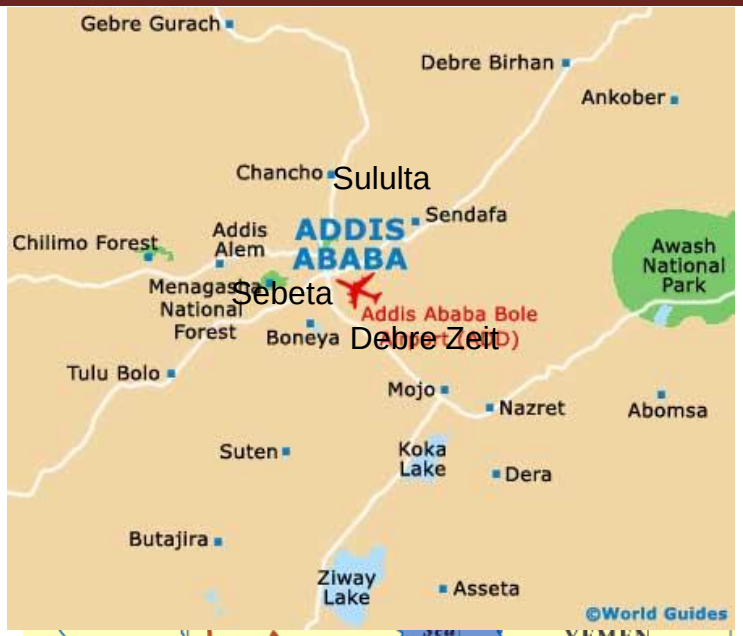
Study locations: the Greater Addis Ababa milk shed

- Includes Addis Ababa, Debre Zeit, Sebeta, Sendafa and Sululta
- It serves as a major milk supplier to urban markets in and around Addis Ababa
- The sector is commercial and uses concentrate feeding



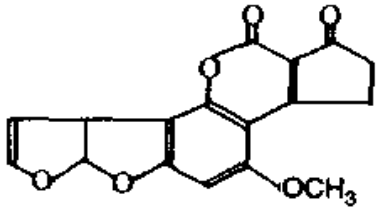
IDDELS (http://www.ideels.uni-bremen.de/highlands.html)

Study methods

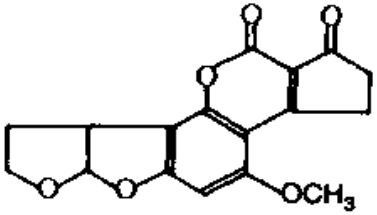
- Study participants:
  - 100 dairy farmers
    - 27 from Addis Ababa, 23 from Debre Zeit, 9 from Sebeta, 31 from Sendafa and 10 from Sululta
  - 5 feed producers
  - 5 feed processors
  - 9 feed traders
- A semi-structured questionnaire was administered to all study participants
- 100 grams of each feed samples were collected

Feed analysis of aflatoxin B1 (AFB1) using enzyme-linked immunosorbent assay (ELISA)

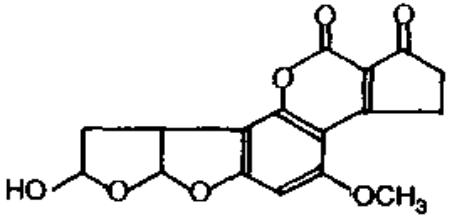
Aflatoxins are difuranocoumarin compounds and vary depending on their chemical structures



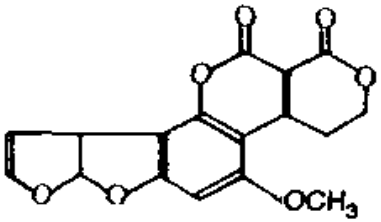
aflatoxin B_1



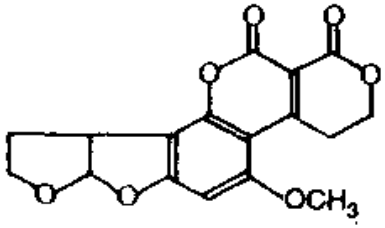
aflatoxin B_2



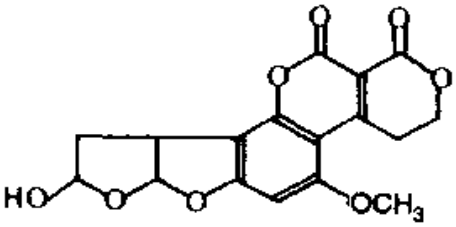
aflatoxin B_{2a}



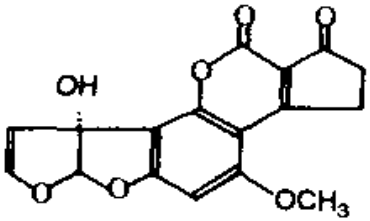
aflatoxin G_1



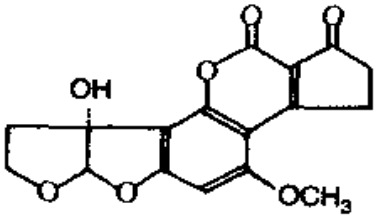
aflatoxin G_2



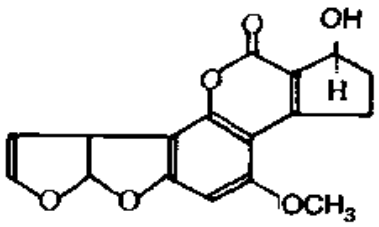
aflatoxin G_{2a}



aflatoxin M_1



aflatoxin M_2



aflatoxicol

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Transformation of AFB1 to AFM1

Animals under the influence of the cytochrome P_{450} oxidase system found in their micro-flora and own cells hydroxylate aflatoxin B_1 (AFB1) to aflatoxin M_1 (AFM1)

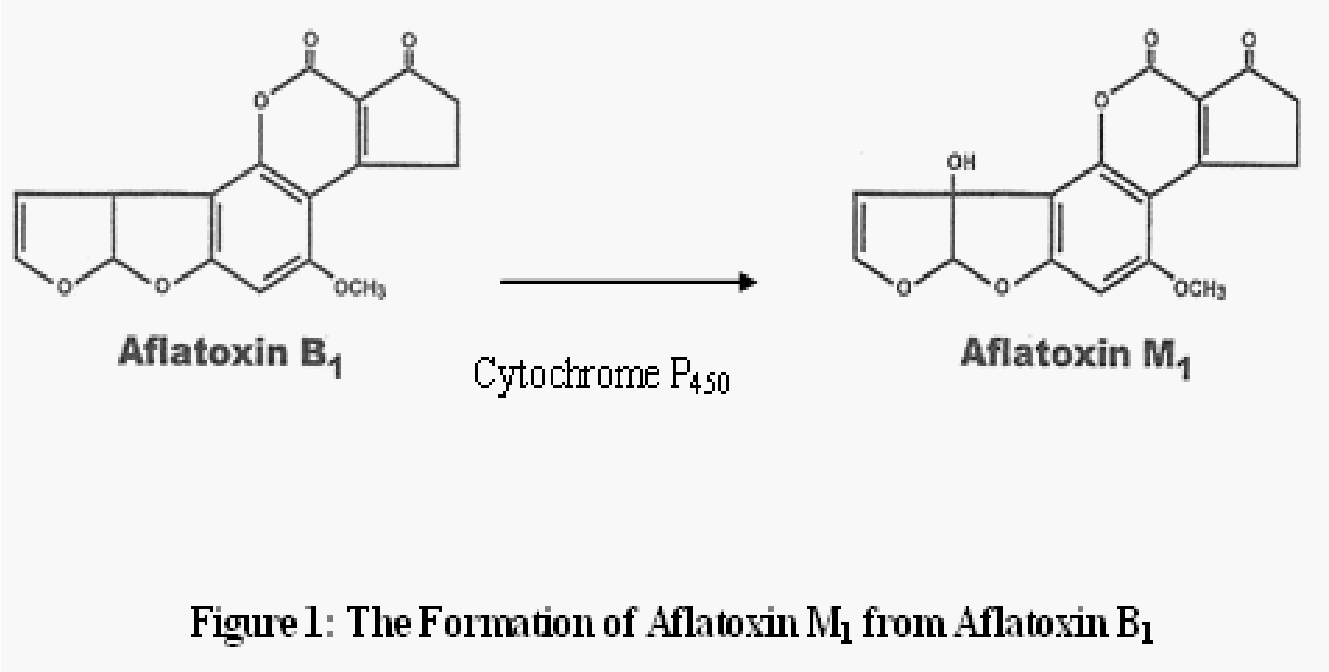
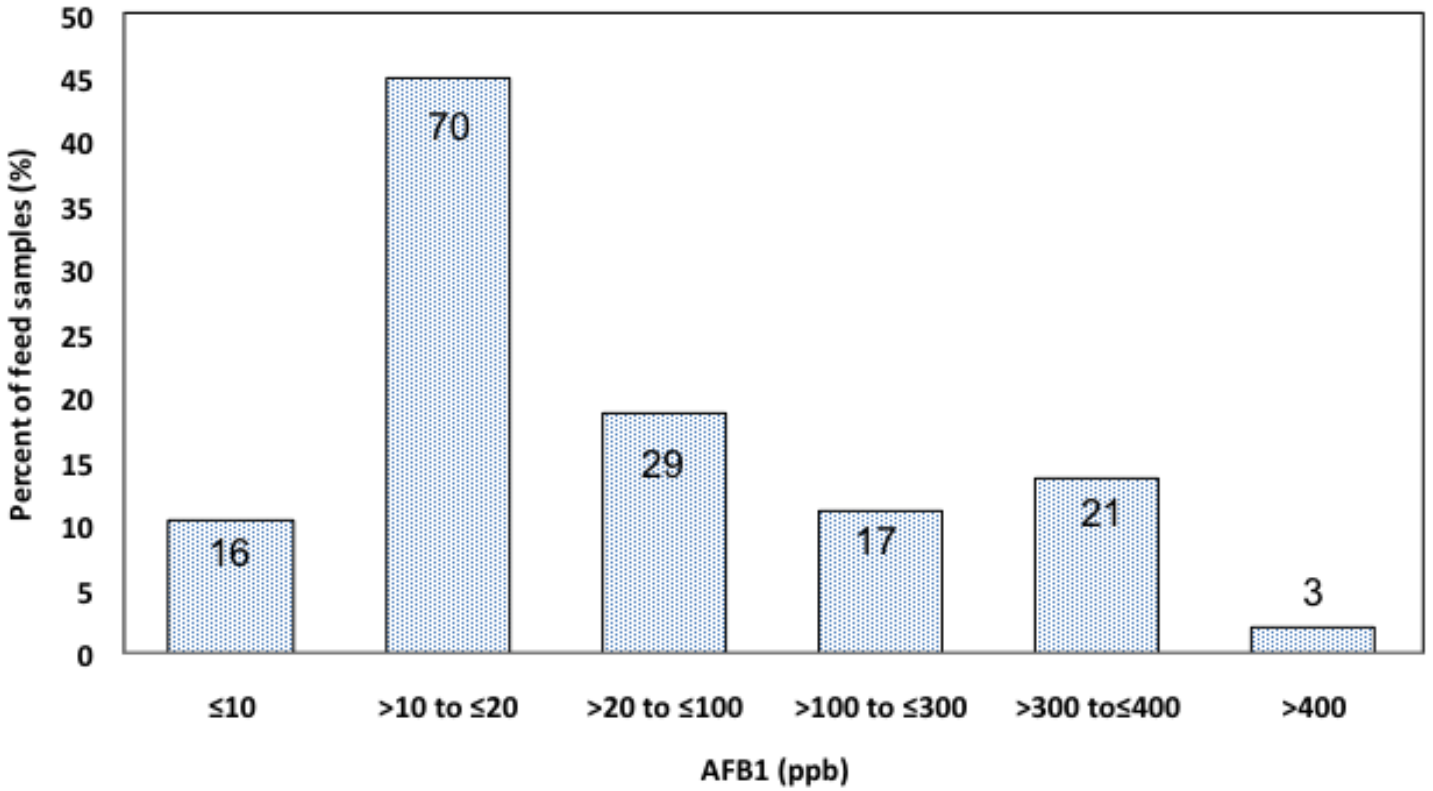


Figure 1: The Formation of Aflatoxin M_1 from Aflatoxin B_1

Results of feed analysis from milk producers, feed manufacturers and traders



Source: Gizachew et al. Food Control 59 (2016) 773-779

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Results of feed analysis

Summary of aflatoxin (AFB1) contamination of feed in the value chain

	AFB1 levels (ppb) in feed samples			
Value chain actor	<5	5-20	20-100	>[c]100
Milk producer	[a]0	61	24	29
	[b]0	54	21	25
Feed manufacturer and trader	[a]0	22	8	12
	[b]0	52	19	29

[a]Number of contaminated samples
[b]Percentage of AFB1 contaminated feed samples
[c]The highest AFB1 concentration was 419 ppb from milk producer

Source: Gizachew et al. Food Control 59 (2016) 773-779

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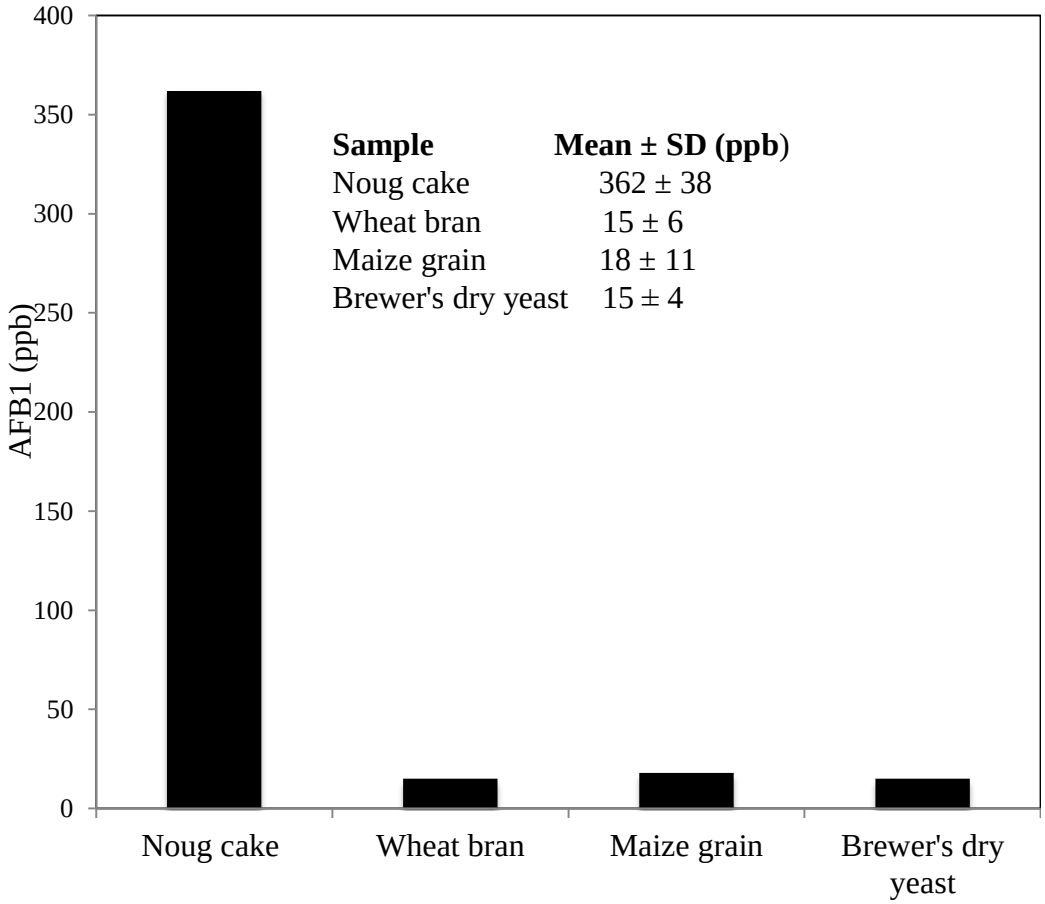
Results of feed analysis

Concentration of AFB1 (ppb) in individual dairy feed ingredients



Source: Gizachew et al. Food Control 59 (2016) 773-779

Results of feed analysis

AFB1 distribution levels and concentrations of AFB1 in feed samples from milk producers per region

Regions	AFB1 levels (ppb) in feed samples				
	[a]No. of samples	[b]<20	[b]20-100	[b]100>	Percentage of 100>
Addis Ababa	27	9	8	10	37
Debre Zeit	23	5	7	11	48
Sebeta	9	6	1	2	22
Sendafa	31	26	4	1	3
Sululta	10	5	1	4	40

[a]Total number of concentrate feed samples from producers per region
[b]Number of contaminated samples

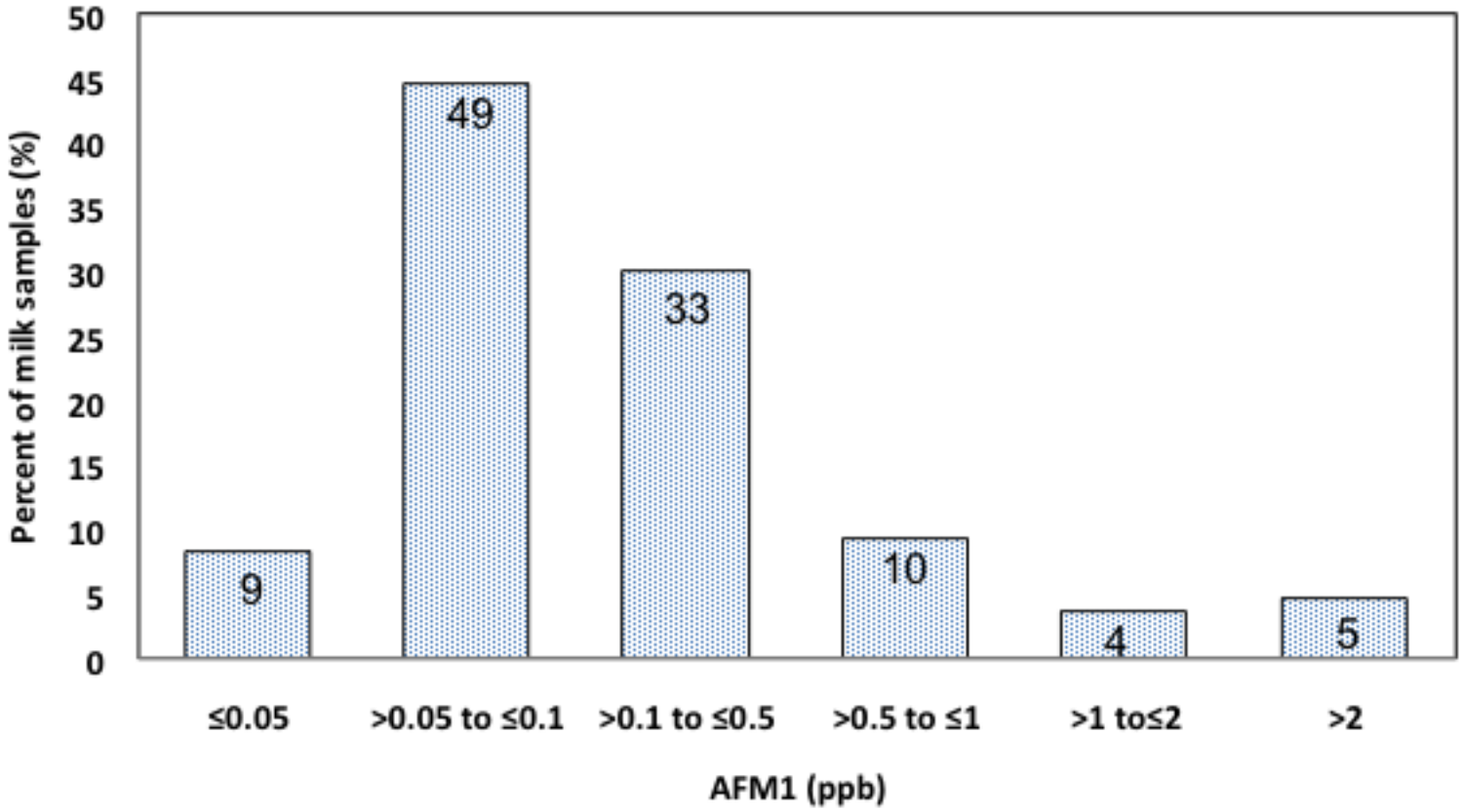
Source: Gizachew et al. Food Control 59 (2016) 773-779

Milk analysis of aflatoxin M1 (AFM1) using enzyme-linked immunosorbent assay (ELISA)

Study methods

- Study participants:
  - 100 dairy farmers
    - 27 from Addis Ababa, 23 from Debre Zeit, 9 from Sebeta, 31 from Sendafa and 10 from Sululta
  - 10 milk collectors
- A semi-structured questionnaire was administered to all study participants
- 50 ml of raw milk samples were collected

AFM1 distribution and percentage in milk from both producers and collectors



Source: Gizachew et al. Food Control 59 (2016) 773-779

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Results of milk analysis from milk producers and collectors

Summary of aflatoxin (AFM1) contamination of milk in the value chain

	AFM1 levels (ppb) in milk samples				
Value chain actor	<0.05	0.05-0.10	0.10-0.50	0.50-1.00	[c]1.00 >
Milk producer	[a,b]8	48	29	7	8
Milk collector	[a]0	3	3	1	3
	[b]0	30	30	10	30

[a]Number of contaminated samples

[b]Percentage of AFM1 contaminated milk samples

[c]The highest AFM1 concentration was 4.98 ppb from milk producer.

Source: Gizachew et al. Food Control 59 (2016) 773-779

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AFM1 distribution levels in milk samples from milk producers per region

Regions	[a]No. of samples	AFM1 levels (ppb) in milk samples [b]<0.05	[b]0.05-0.1	[b]0.1-0.5	[b]0.5>	Percentage of 0.5>
Addis Ababa	27	3	8	12	4	14.8
Debre Zeit	23	2	8	9	4	17.4
Sebeta	9	0	6	1	2	22.2
Sendafa	31	3	22	5	2	6.4
Sululta	10	0	3	3	4	40

[a]Total number of milk samples from producers per region
[b]Number of contaminated samples

Source: Gizachew et al. Food Control 59 (2016) 773-779

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Correlation between high-level AFB1 and AFM1 levels for samples collected from milk producers

Region	Feed samples used by milk producers	AFB1 (ppb)	AFM1 (ppb)
Debre Zeit	Wheat bran and noug seed cake mix	405	4.98
	Maize and sweet pea grain mix	30	
Sululta	Wheat bran, maize grain and noug seed cake mix	300	4.79
Sendafa	Wheat bran and sweet pea hull mix	14	2.93
Addis Ababa	Wheat bran, sweet pea hull and noug seed cake mix	72	2.92
Sululta	Wheat bran, maize grain, and noug seed cake mix	264	2.57
Debre Zeit	Wheat bran, maize grain, and noug seed cake mix	274	2.16
	Maize grain	7.5	
	Beer byproduct	7.7	
Debre Zeit	Wheat bran and noug seed cake mix	356	1.62
	Maize grain	8.7	
Sululta	Wheat bran, maize grain, calcium and noug seed cake mix	17	1.23

Source: Gizachew et al. Food Control 59 (2016) 773-779

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Why does noug cake have high aflatoxin contamination?

- Identify the types of *Aspergillus* fungi that grow on noug seed and noug cake
- Study conditions that are conducive to growth and toxin production on noug and noug seed cake

Noug cake

- Investigate storage conditions of noug seed and cake

Why are there regional differences in level of aflatoxin contamination within the Greater Addis Ababa milk shed?

- Study the differences in climate, storage conditions and the proportion of noug cake in the feed

- Study aflatoxin contamination in other milk sheds in Ethiopia

Aflatoxin contamination of poultry feed

- Study poultry feed for aflatoxin contamination
- Study its effect on egg production and impaired growth

Effect of aflatoxin in broiler growth at 42 days.
T1 – control
T2– 3 ppm of aflatoxin
T6 – control + ADS (0.2%)
(Source: Agranco Corp. USA)

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Chemical detoxification of aflatoxin (AFB1) in feed

- Treatment of aflatoxin-contaminated feed samples with salts such as sodium bicarbonate and sodium bisulphite
  - Study the effect of concentration and temperature of the salt solution
  - Study mixture of salts treatment to maximize the detoxification

How much do we know about the impact of aflatoxin on human health in Ethiopia?

- Together with the Ministry of Health, study aflatoxin in human urine and breast milk
- This might reveal the level of aflatoxin exposure in people who have been consuming the contaminated milk

Conclusions

- High level contamination of aflatoxin in feed (AFB1) and in milk (AFM1)
- Noug (*Guizotia abyssinica*) cakes are widely used in the greater Addis Ababa milk shed as cattle feed and have been found to be highly contaminated with AFB1
- Further studies on the impact of aflatoxin on both human and animal health in Ethiopia are required
- Intervention studies, including chemical detoxification of the feed, are necessary to minimize aflatoxin contamination along the value chain

Acknowledgements

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