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Aflatoxin Contamination and Rancidity in Locally Processed Commercial Fish Feeds and Ingredients along the Value Chain in Wakiso and Kampala Districts, Uganda

Joseph Magala[1*], David Kahwa[2], Edward Nyatia[3], Timothy Kasule[4], Fortunate Natwijuka[5]

[1]National Agricultural Research Organization, Bulindi Zonal Agricultural Research and Development Institute, Hoima, Uganda
[2]Department of Wildlife and Animal Resource Management, School of Veterinary Medicine and Animal Resources, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda
[3]Department of Biomolecular Resources and Biolaboratory Sciences, School of Biosecurity Biotechnical and Laboratory Sciences, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda
[4]National Livestock Research Institute, Namulonge, Uganda
[5]Medical Research Council/Uganda Virus Research Institute & London School of Hygiene and Tropical Medicine, Entebbe, Uganda
Email: *magalajoseph37@gmail.com

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Abstract

Aflatoxin contamination and rancidity in locally processed commercial fish feeds and ingredients along value chains is a Public and Animal health hazard. The study established the level of aflatoxin contamination, peroxide value (PV), Anisidine value (AnV), and their associated factors at storage areas among farmers, processors, traders, factories, and landing sites in the Wakiso and Kampala districts. The value chain actors were purposively selected in a cross-sectional study based on access to the feed store and the use of locally processed commercial fish feeds on farms. Data collected were statistically analyzed in SPSS version 20. All the samples (45) were positive for aflatoxin contamination and PV with 51% (23/45) of samples being contaminated with "above acceptable" aflatoxin levels and 66.6% (30/45) of samples with "above acceptable" PV. The overall percentage of "above acceptable" AnV was 11/29 (37.9%). Samples from factories were within acceptable contamination levels. Multivariate logistic regression analysis revealed no significant difference between aflatoxin contamination, peroxide, and Anisidine value with storage factors for locally processed commercial fish feeds and ingredients. The study recommended the purchase of fish feeds from factories and a larger study on storage factors responsible for aflatoxin contamination and rancidity in fish feeds in Uganda.

Keywords

Aflatoxin Contamination, Rancidity, Locally Processed, Fish Feeds and Ingredients

1. Introduction

The rapid rise of aquaculture has been fueled by the global decline in capture fisheries and the ongoing need to feed a continually expanding human population [1]. Aquaculture currently accounts for more than half of all fish ingested directly by humans. As global capture fisheries stagnate and worldwide demand for fish rises, the proportion will increase in the next decades. The human population has expanded beyond 7.1 billion people, and the reliance on farmed fish as a source of protein will as well grow [2].

Aquaculture production in Africa is 95% small-scale and still developing mostly in a few countries. Sub-Saharan Africa (SSA) contributes only 0.63% of the global production. Uganda tops the list of ten countries in SSA with the fastest rates of fish and aquaculture production growth and exports outside the region [3]. The existence of freshwater bodies such as lakes, rivers, and swamps, has boosted economic activity [4]. Aquaculture is performed virtually everywhere in the country, with Tilapia and North African catfish being the most farmed fish species [5]. Despite scientific advancements in aquaculture, fish feed technology has remained one of Africa's least developed aquaculture value chain sectors [6].

Fish nutrition with feed quality also directly impacts fish health and output [7]. The producers have resorted to the utilization of imported fish feeds from European countries. However, this solution has resulted in extremely high farming costs and increased total variable costs for aquaculture production. Feed accounts for more than 60% of total variable costs in aquaculture production. Consequently, producers have resorted to the use of non-conventional and locally available fish feed ingredients including agro-industrial by-products rice bran, maize bran, fishmeal, sunflower seedcakes, soybean cake, cassava, cottonseed cake among others [8]. The locally produced commercial fish feeds in Uganda have received several quality-related complaints from fish farmers [9]. Research on the utilization of locally available and produced low-cost feed components is very vital for the development of the aquaculture sector [4].

The plant-based ingredients are prone to contamination by moulds during pre-harvest and due to poor post-harvest treatments like unfavourable storage conditions and infrastructure. Prolonged storage, high temperature, and humid conditions are some of the factors that predispose the ingredients to fungal development and production of attendant mycotoxins that compromise feed quality to adversely affect the health of animals and humans [10].

Aflatoxins are fungal secondary metabolites that are highly toxic, and carcinogenic mostly generated by Aspergillus species. These species are prevalent in most soils and they infect grains and other farm products used in animal feed production both at pre-harvest and post-harvest, producing aflatoxins when conditions are favorable. There are at least 13 different forms of aflatoxins, but the most important are aflatoxins B1, B2, G1, and G1 with B1 being the most poisonous and widespread [11].

Lipids are significant constituents in fish feeds, because they help with palatability, satiety, and nutrition [12]. However, metabolic interaction between lipids and oxygen may result in the generation of short-chain molecules from long-chain fatty acids by a process referred to as rancidity. With the presence of unfavorable storage conditions, these chemical processes can produce highly reactive molecules in rancid fats and oils, which cause disagreeable aromas and flavor [13]. Chemical processes deplete feed of nutrients and in some situations, rancidity and vitamin degradation occur very quickly. Due to the high degree of unsaturation, the fatty acids are particularly susceptible to oxidative rancidity [14].

Feed manufacturers attempt to prevent oxidation in lipid sources such as fish oil by stabilizing them with antioxidants. However, typical antioxidants like Ethoxyquin, Butylated Hydroxy Anisole (BHA), and Butylated Hydroxy Toluene (BHT) are sacrificial in their protection of the oil. When depleted, free radicals already present in the oil begin to react with unsaturated fatty acid components, and the oxidation process begins [15]. Rotating the feed inventory as rapidly as possible is the only effective approach for preventing fish feeds from becoming rancid before it is consumed. This can be accomplished easily with feeds that are fed in high volume [16]. Contamination of fish feed has effects on fish among others poor development evidenced by reduced daily weight gain and the occurrence of gross and microscopic lesions leading to economic losses due to low output, morbidity, mortality, and poor quality of fish and fish products [17]. The current research was aimed at assessing aflatoxin contamination peroxide value (PV), anisidine value (AnV), and their associated factors in locally processed commercial fish feeds and ingredients along the value chain in the Wakiso and Kampala districts of Uganda.

2. Materials and Methods

2.1. Data Collection Management and Analysis

A handful of locally sourced fish feeds and ingredients were collected using a clear zip-lock bag among the fish feed and ingredients stores with, fish farmers, traders, processors, factories, and landing sites in Kampala and Wakiso districts. The sample details were recorded on sticky notepaper and inserted inside the plastic jacket. Questionnaires were used to assess the storage factors at storage areas for locally processed fish feeds and ingredients. A feed thermometer was used to evaluate the temperature of the feed and ingredients in storage areas. The collected samples were analyzed in Chemiphar laboratories, Kansanga

Kampala for aflatoxin contamination, peroxide& Anisidine values following the procedures by the Association of Official Analytical Chemists (AOAC).

Data collected were recorded in a Microsoft Excel 2007 spreadsheet. The data for the first 2 objectives were analyzed using **SPSS version 24**. Data for the associated factors were analyzed using **STATA version 13** to establish the bivariate and multivariate logistic regression tests for storage factors associated with aflatoxin contamination and rancidity in locally processed aquaculture feeds and ingredients.

2.2. Laboratory Analysis for Aflatoxin Contamination (PPB), Peroxide Value (PV) & Anisidine Value (AnV) for Locally Processed Fish Feeds & Ingredients

2.2.1. Aflatoxin Contamination Analysis for Locally Processed Fish Feeds & Ingredients

The study used Immunological Methods, Enzyme Link Immunosorbent Assay (ELISA). It is one of the laboratory procedures for quantifying the actual amounts of aflatoxin. It can detect and quantify the presence of an antigen (aflatoxin) in a sample using an enzyme-labeled toxin and antibodies specific to aflatoxin [18]. The study approached laboratory analysis of samples using RIDA SCREEN FAST®, which is a competitive aflatoxin detection kit performed on a plastic 48 micro wells coated plate. The kit was selected mainly due to its attributed accuracy and consistency, as its results are comparable with the published HPLC method.

To 50 g of sample, 10 g NaCl and 250 ml (Methanol: Water, 70:30 v/v) were added to a blending jar. The mixture was then filtered through Whatman 1 and 50 μl aliquot pipetted. One hundred microliters of the sample were added to 200 μl conjugate into each color-coated dilution well and stirred. The content (100 μl) was transferred into an antibody-coated well and incubated for 5 - 15 minutes. The contents in the wells were discarded and washed with deionized water/buffer solution. The wells were tap dried on an absorbent paper towel and 100 μl substrate was added to each well and incubated for 5 minutes. A stop solution (100 μl) was added to each well.

2.2.2. Peroxide Value in Locally Processed Fish Feeds & Ingredients

This analysis offers the advantage of being fast and indicates the first products of lipid oxidation. The peroxide value (PV) is defined as the reactive oxygen contents expressed in terms of mill equivalents (meq) of free iodine per kilogram of fat. It is determined by titrating iodine liberated from potassium iodide with sodium thiosulphate solution. Titration methods have been documented to be widely used for the analysis of primary rancidity in fats and oils. Peroxide values of fresh oils are less than 20 meq/kg. When the peroxide value is above 20 meq/kg, a rancid taste is noticeable.

Procedure

Two grams (02 g) of oil dissolved in10ml of an Acetic acid/Isooctane (3:2, v/v). The solution was gently mixed and 01 ml of 0.5 M Potassium Iodide solution was added as an indicator. The mixture was incubated for 10 minutes in the dark and diluted with 20 ml of distilled water. The mixture was titrated with 0.01 M Sodium thiosulfate in presence of starch solution (1%,1 ml) until the dark blue color disappeared.

2.2.3. Anisidine Value (AnV)

This refers to 100 times the contribution of the extinction of 1g oil in 100 ml solvent and Anisidine reagents read at 350 nm. Anisidine value involves the measure of alpha-beta unsaturated aldehydes of oils. The secondary oxidation products formed are upon the breakdown of the peroxides and are responsible for off-flavors.

Two 02 grams of oil dissolved in 50 ml of an ethanol/ether mixture (1:1, v/v). This solution is gently mixed and 01 ml of 1% phenolphthalein solution is added as an indicator. The mixture was titrated with 0.1 M Ethanolic Potassium Hydroxide until its color changed to light pink.

The AV is expressed as milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of oil. The resulting solution was well mixed, and its absorbance was measured at 350 nm. The AV was calculated using a pre-plotted calibration curve.

N.B: Each sample analyzed individually in triplicate for aflatoxin contamination, peroxide, and anisidine values.

3. Results

3.1. Aflatoxin Contamination in Locally Processed Commercial Fish Feeds & Ingredients

All samples (100%, n = 45) examined in the laboratory in the current study were contaminated with aflatoxin at varying levels. The overall contamination revealed 51% (23/45) of samples beyond acceptable aflatoxin level (20 ppb/kg). For individual value chain activity, 65% (13/20) of samples from farmers, 30% (3/10) among processors, 60% (6/10) among traders, and 50% (1/2) of samples from landing sites were beyond acceptable aflatoxin level. All samples from the factory were within acceptable aflatoxin contamination levels as shown in Table 1.

3.2. Rancidity in Locally Processed Commercial Fish Feeds and Ingredients along the Value Chain

3.2.1. Peroxide Values for Locally Processed Commercial Fish Feeds and Ingredients

All the samples (100%, n = 45) were tested positive for peroxide values at varying levels. The overall percentage of samples beyond acceptable peroxide value was 30/45 (66.6%). The individual value chain revealed, that 65% (13/20) of samples from farmers, 50% (5/10) of samples from processors, 90% (9/10) of samples from traders, and 100% (2/2) of samples from landing sites were above acceptable peroxide value (20 meq/kg). All samples from the factory had acceptable peroxide values (<20 meq/kg) as shown in Table 2.

Table 1. Distribution of aflatoxin contamination in locally processed commercial fish feeds and ingredients along the value chain.

Value Chain Activity	No. of samples analyzed	No. of samples positive	% of samples beyond acceptable levels (20 ppb)	Range (ppb)		
				Low	High	Mean ± SE
Farmers	20	20	13 (65%)	4.80	45.90	24.63 ± 2.67
Processor	10	10	3 (30%)	5.90	37.50	17.68 ± 3.07
Trader	10	10	6 (60%)	10.60	34.10	23.10 ± 2.47
Factory	3	3	-	6.30	11.90	8.76 ± 1.65
Landing site	2	2	1 (50%)	31.0	29.30	21.15 ± 8.15

Table 2. Distribution of peroxide values in locally processed commercial fish feeds and ingredients along the value chain.

Value Chain Activity	No. of samples analyzed	No. of +ve samples	% of samples >20 meq/kg	Range (meq/kg)		
				Low	High	Mean ± SE
Farmers	20	20	13 (65%)	1.93	39.60	24.22 ± 2.67
Processor	10	10	5 (50%)	14.51	30.20	21.94 ± 3.07
Trader	10	10	9 (90%)	15.40	44.24	27.07 ± 2.45
Factory	3	3	0 (0%)	16.30	19.60	18.13 ± 0.97
Landing site	2	2	2 (100%)	30.40	39.80	35.10 ± 4.70

3.2.2. Anisidine Value (AnV) for Locally Processed Fish Feeds and Ingredients

Anisidine value (AnV) was analyzed only for the samples that tested positive for peroxide value (PV), 64.4% (29/45) of the total samples collected. All the samples (100%, n = 29) were positive for Anisidine value. The overall percentage of samples above acceptable anisidine value was 37.9%. The individual value chain activity revealed that 46.1% (6/13) of samples from farmers, 33% (3/9) of samples from traders, 20% (1/5) of samples from processors, and 50% (1/2) of samples from the landing were above acceptable anisidine value. No sample from the factory qualified for analysis of anisidine value as shown in Table 3.

3.3. Socio-Demographic Characteristics of the Respondents along the Value Chain for Locally Processed Commercial Fish Feeds and Ingredients

Males (75.6%) dominated the study. Wakiso district contributed a high number of study respondents and units (55.6%). The value chain activity revealed the dominance of farmers during the study (44.4%). Many study respondents were aged between 40 - 49 years (35.6%). Value chain actors with a secondary level of education dominated the study (42.2%) as shown in Table 4.

Table 3. Distribution of anisidine Value for locally processed commercial fish feeds and ingredients along the value chain.

Value Chain Activity	(n) samples analyzed	No. of +ve Samples	% of samples > 20	Range		
				Low	High	Mean ± SE
Farmers	13	13	6 (46.1%)	13.28	26.16	18.93 ± 1.13
Processor	5	5	1 (20%)	14.42	20.33	17.89 ± 1.01
Trader	9	9	3 (33%)	15.40	44.24	19.88 ± 1.93
Landing site	2	2	1 (50%)	14.45	26.49	20.47 ± 6.02

Table 4. Demographic characteristics of the respondents along the locally processed commercial fish feeds and ingredients value chain.

Characteristic	Category	Frequency	Percentage (%)
Sex	Male	34	75.6
	Female	11	24.4
District	Kampala	20	44.4
	Wakiso	25	55.6
Value Chain Activity	Farmers	20	44.4
	Processor	10	22.2
	Trader	10	22.2
	Factory	3	6.7
	Landing site	2	4.4
Age	Below 20	1	2.2
	20 - 29	10	22.2
	30 - 39	13	28.9
	40 - 49	16	35.6
	50 and above	3	6.7
Education Level	Non-formal	1	2.2
	Primary	15	33.3
	Secondary	19	42.2
	Tertiary	10	22.2

3.4. Distribution of Storage Period for Locally Processed Commercial Fish Feeds and Ingredients along the Value Chain

The majority (40%) of farmers stored fish feeds and ingredients for 0-to 2 weeks. Thirty percent (3/10) of processors stored fish feeds and ingredients between 3 - 4 weeks and above 6 weeks of storage. Sixty percent (6/10) of traders stored fish feeds and ingredients above 6 weeks. More than 67% (2/3) of factories stored fish feeds and ingredients above 6 weeks. Silverfish at the landing site was stored for a period not exceeding two weeks as shown in **Table 5**.

Table 5. Storage period of locally processed commercial fish feeds along the value chain activity.

V C A	Storage period in weeks			
	0 - 2 weeks	3 - 4 weeks	5 - 6 week	>6 weeks
Farmer	8 (40%)	7 (35%)	0 (0%)	5 (25%)
Processor	2 (20%)	3 (30%)	2 (20%)	3 (30%)
Trader	1(10%)	2 (20%)	1 (10%)	6 (60%)
Factory	2 (67.7%)	0 (0%)	0 (0%)	1 (33.3%)
Landing site	2 (100%)	0 (0%)	0 (0%)	0 (0%)

V.C.A: Value Chain Activity.

3.5. Factors Associated with Aflatoxin Contamination, Peroxide, and Anisidine Value for Locally Processed Commercial Fish Feeds and Ingredients during Storage

The definition of acceptable and not acceptable levels used to evaluate factors in the storage area is based on the observations and findings.

Factors associated with with aflatoxin contamination, peroxide and anisidine value for locally processed commercial fish feeds and ingredients during storage.

1) Temperature

Acceptable	Not acceptable
15˚C - 27˚C for dry fish feeds and ingredients	>27˚C for dry fish feeds and ingredients

2) Sunlight

Acceptable	Not acceptable
Fish feeds and ingredients are away from sunlight.	Fish feeds and ingredients accessed by sunlight.
Opaque/translucent packaging materials	Packages that can't protect the feeds and ingredients from sunlight

3). Packaging material

Acceptable	Not acceptable
Laminating packaging material	Non-laminating packaging material
Feeds are in whole bag	Feeds kept in torn bags.

4) Storage Period

Acceptable	Not acceptable
0 - 6 weeks of storage (Complete feeds)	>6 weeks storage (Complete feed)
0 - 8 weeks of storage for whole ingredients	>8 weeks of storage for whole ingredients
0 - 12 weeks of storage for pellets and powder.	>12 weeks of storage (factory feeds)

5) Rodents & Vermin

Acceptable	Not Acceptable
No feeds are damaged by rodents	Damaged feed sacs by rodents
Absence of rodents in the store	Presence of vermin & rodents
Absence of insects like weevils in store	Infestation of fish feeds and ingredients
Availability of meshes and rat guards	No measures to protect rodents

6) Leak proof store

Acceptable	Not acceptable
The roof of the store has no wholes, prevents rain water into stored feeds	The roof of the store has no wholes, prevents rain water into stored feeds
The nature of the walls for the store	The nature of the walls for the store

7) Staking

Acceptable	Not acceptable
The feeds are stacked on pallets or other wooden material.	The feeds not stacked on pallets or other wooden material
Feeds are away from the store walls	Feeds piled reach the store walls

3.5.1. Distribution of Aflatoxin Contamination Levels with Storage Factors for Locally Processed Commercial Fish Feeds and Ingredients

The majority of fish feeds and ingredients (58%) with Not acceptable storage temperature had aflatoxin levels > 20 pp. More than 51% (21/41) of fish feeds and ingredients with access to rodents and vermin were above acceptable aflatoxin levels. Other storage factors are summarized in Table 6.

3.5.2. Association between Aflatoxin Contaminations with Storage for Locally Processed Fish Feeds and Ingredients

The association between aflatoxin contamination and storage factors was assessed using chi-square with a p-value set at ≤0.2. Only the storage period was significant (OR = 0.4, 95% CI: 0.11 - 1.48). Temperature, Rodents & vermin, and leak-proof storage area were not statistically significant at a p-value ≤0.2 as shown in Table 7.

3.5.3. Multivariate Analysis for "Above Acceptable" Aflatoxin Contamination Levels with Storage Factors for Locally Processed Commercial Fish Feeds and Ingredients

All the storage factors from the bivariate analysis were fit for multivariate analysis. Feeds from storage areas with access to rodents and not acceptable temperature were found to be associated with aflatoxin contamination (OR = 2.4, 95% CI: 0.03 - 1.21 & OR = 1.8, 95% CI: 0.35 - 9.66) respectively although were not statistically significant at p-value set at 0.05. Fish feeds and ingredients with access to rodents and vermin, not acceptable stacking, and the unacceptable leak-proof store was associated with aflatoxin contamination at above acceptable levels though were not statistically significant as shown in Table 8.

Table 6. Distribution of aflatoxin contamination levels within storage factors for locally processed commercial fish feeds and ingredients.

Storage factor	Category	+ve samples (n)	Aflatoxin > 20 ppb
Leak proof store	Acceptable	4	3 (75%)
	Not acceptable	41	20 (48.78%)
Temperature	Acceptable	33	16 (48.48%)
	Not acceptable	12	7 (58.33%)
Storage period	Acceptable	31	18 (58.06%)
	Not acceptable	14	5 (35.71%)
Stacking of feeds	Acceptable	14	9 (64.29%)
	Not acceptable	31	14 (45.16%)
Vermin & rodents	Acceptable	4	2 (50%)
	Not acceptable	41	21 (51.22%)

Table 7. Association between of aflatoxin contamination with storage factors for fish feeds and ingredients.

Storage factor	Category	Odds Ratio	95% CI	p-value
Temperature	Acceptable	1	(0.39 - 5.65)	
	Not acceptable	1.5		0.56
Storage period	Acceptable	1	(0.11 - 1.48)	
	Not acceptable	0.4		**0.17***
Rodents &Vermin	Acceptable	1	(0.13 - 8.18)	
	Not acceptable	1.1		0.963
Leak proof store	Acceptable	1	(0.03 - 3.31)	
	Not acceptable	0.3		0.337
Stacking	Acceptable	1	(0.12 - 1.68)	
	Not acceptable	0.5		0.239

Table 8. Multivariate analysis for "above acceptable" aflatoxin contamination levels with storage factors for locally processed commercial fish feeds and ingredients.

Storage factor	O R	95%CI	P-value
N. A Temperature	1.8	(0.35 - 9.66)	0.474
N.A Storage Period	0.2	(0.03 - 1.21)	0.082
N.A Rodents & Vermin	2.4	(0.24 - 24.55)	0.454
N.A Stacking	0.7	(0.17 - 2.99)	0.652
N.A Leak proof store	0.2	(0.01 - 2.60)	0.211

N.A: Not Acceptable storage factor.

3.6. Distribution of Rancidity at Storage Areas for Locally Processed Commercial Fish Feeds and Ingredients

3.6.1. Distribution of Peroxide Values with Storage Factors for Locally Processed Commercial Fish Feeds and Ingredients

More than 58% (18/31) of samples collected from acceptable packaging material were above acceptable peroxide value. More than 78% (11/14) of samples from unacceptable packaging material were above acceptable peroxide value. In addition, 75% (9/12) of fish feeds and ingredients with no acceptable temperature value (>27˚C) were above acceptable peroxide value. Generally, samples from not acceptable storage conditions exhibited high levels of contamination as compared to those from acceptable storage conditions as shown in Table 9.

3.6.2. Association between Storage Factors and above Acceptable Peroxide Values for Locally Processed Commercial Fish Feeds and Ingredients

The association between storage factors and peroxide value was with a p-value set at ≤0.2. Only the storage period was a statistically significant factor (OR = 2.6, 95% CI: 0.61 - 11.43). Temperature, sunlight, and packaging material were not statistically significant at a p-value ≤ 0.2 as shown in Table 10.

3.6.3. Multivariate Logistic Regression Analysis for Peroxide Value with Storage Factors for Locally Processed Commercial Fish Feeds and Ingredients

From bivariate analysis, variables with p-value ≤ 0.2, storage period, and those with biological plausibility (as it was for all the factors) were run for multivariate logistic regression. Samples with the above acceptable storage period were found to be associated (OR = 2.43, 95% CI: 0.49 - 11.97) though the association was not statistically significant as shown in Table 11.

3.6.4. Distribution of Anisidine Values across Potential Storage Factors in Locally Processed Commercial Fish Feeds and Ingredients

More than 33% (6/18) of sample storage areas with no access to sunlight were above acceptable anisidine levels. More than 45% (5/11) of fish feeds and samples from storage areas with access to sunlight were above acceptable anisidine value. More than 55% (5/9) of samples from storage areas beyond acceptable storage temperature were beyond acceptable anisidine value. In addition, more than 45% (5/11) of fish feeds and samples from storage areas with access to sunlight were above acceptable anisidine value. Other factors evaluated in storage areas are shown in Table 12.

3.6.5. Association between above Acceptable Anisidine Values with Storage Factors for Locally Processed Commercial Fish Feeds and Ingredients

In bivariate analysis, the association between the above acceptable peroxide value with storage factors was assessed using chi-square with a p-value set at ≤0.2. Storage temperature was statistically significant (OR = 2.9 95% CI: 0.57 - 14.82). Storage period sunlight and packaging material were not statistically significant at p-value ≤ 0.2 as shown in Table 13.

3.6.6. Multivariate Logistic Regression for Anisidine Values and Storage Factors in Locally Processed Commercial Fish Feeds and Ingredients

From the bivariate analysis above, variables with p-values at ≤0.2, and those with biological plausibility (as was the case for all the factors) were analyzed for multivariate logistic regression. Feeds with not acceptable temperatures were associated with above acceptable anisidine value (OR = 7.329, 95% CI: 0.66 - 78.39). Feeds exposed to sunlight in storage areas were associated with the above acceptable anisidine value (OR = 1.14, 95% CI: 0.36 - 7.77). Feeds from improper packaging material were associated with beyond acceptable anisidine value (OR = 0.8, 95% CI: 0.09 - 2.37). Feeds above acceptable storage period were 0.1 times more likely to have above acceptable anisidine value (OR = 0.173, 95% CI: 0.01 - 2.21). The results revealed no significant difference in independent factors with anisidine value (P > 0.05) as shown in Table 14.

Table 9. Distribution of peroxide values between storage factors for locally processed commercial fish feeds and ingredients.

Factor	Category	No. of +ve samples	P.V > 20 meq/kg
Sun light	Acceptable	30	18 (60%)
	Not acceptable	15	11 (73.3%)
Temperature	Acceptable	33	20 (60.6%)
	Not acceptable	12	9 (75%)
Packaging Material	Acceptable	31	18 (58.8%)
	Not acceptable	14	11 (78.6%)
Storage period	Acceptable	12	7 (58.3%)
	Not acceptable	33	22 (66.6%)

Table 10. Association between storage factors and "above acceptable" peroxide values for locally processed commercial fish feeds and ingredients.

Storage factor	Category	OR	95% CI	p-value
Temperature	Acceptable	1	(0.44 - 8.58)	
	Not acceptable	2.0		0.38
Sunlight	Acceptable	1	(0.47 - 7.13)	
	Not acceptable	1.8		0.38
Packaging material	Acceptable	1	(0.37 - 5.55)	
	Not acceptable	1.4		0.61
Storage period	Acceptable	1	(0.61 - 11.43)	
	Not acceptable	2.6		**0.19***

Table 11. Multivariate logistic regression analysis for peroxide value with storage factors for locally processed commercial fish feeds and ingredients.

Storage factor	OR	95% CI	P-value
N. A Temperature	1.3	0.24 - 6.63	0.779
N.A Sunlight	1.7	0.35 - 8.04	0.516
N.A Packaging material	0.8	0.18 - 4.04	0.834
N.A Storage period	2.4	0.49 - 11.97	0.274

NA: Not acceptable level of factor.

Table 12. Distribution of anisidine values between storage factors for locally processed commercial fish feeds and ingredients.

Factor	Category	No. of samples	AnV (>20)
Sunlight	Acceptable	18	6 (33.3%)
	Not acceptable	11	5 (45.5%)
Temperature	Acceptable	20	6 (30%)
	Not acceptable	9	5 (55.5%)
Packaging Material	Acceptable	7	3 (42%)
	Not acceptable	22	8 (36.6%)
Storage period	Acceptable	18	8 (44.4%)
	Not acceptable	11	3 (27.2%)

Table 13. Association of above acceptable anisidine value with storage factors for locally processed commercial fish feeds and ingredients.

Storage factor	Category	Odds Ratio	95% CI	p value
Temperature	Acceptable	1	(0.57 - 14.82)	**0.197***
	Not acceptable	2.9		
Sunlight	Acceptable	1	(0.36 - 7.77)	0.515
	Not acceptable	1.7		
Packaging material	Acceptable	1	(0.13 - 4.30)	0.758
	Not acceptable	0.8		
Storage period	Acceptable	1	(0.09 - 2.37)	0.359
	Not acceptable	0.5		

Table 14. Multivariate logistic regression for anisidine value in locally processed commercial fish feeds and ingredients.

Storage factor	Odds ratio	95% CI	p-value
N. A Temperature	7.2	0.67 - 78.39	0.103
N.A Sunlight	1.1	0.16 - 8.36	0.895
N.A Packaging material	0.9	0.09 - 10.15	0.972
N.A Storage period	0.2	0.01- 2.21	0.178

N. A: Not Acceptable level of storage factor.

4. Discussion

The need to carry out a study on aflatoxin contamination and rancidity was based on several previous studies in Uganda that have demonstrated the presence and impact of aflatoxin in human food, and livestock feeds with limited documentation in aquaculture. This study was purposely to assess aflatoxin contamination, rancidity, and their associated factors at storage areas for locally processed commercial fish feeds and ingredients along the value chain in Uganda.

The overall percentage and mean "above acceptable" aflatoxin contamination values in the current study were 51% & 21.53 ppb respectively. The contamination in the current study conformed with findings from Nakuru, Kenya (56%) [19]. However, lower aflatoxin contamination beyond the acceptable level (32%) was revealed in maize bran and maize used as animal feed in Northern Tanzania [20]. The aflatoxin contamination in the current study is official to improper storage facilities observed during sample collection.

Except in factories, individual value chain activity revealed that 65% of samples were "above acceptable" aflatoxin-contaminated levels in farmers, 60% in traders, 50% in landing sites, and 30% in processors. The study revealed no significant difference in aflatoxin contamination with value chain activities. A similar study in Kenya revealed a slightly higher prevalence (67%) in farmers [21]. Nevertheless, lower contamination (36.5%) was reported in Croatia [22]. The high percentage of "above acceptable "aflatoxin contamination levels among farmers in the current study could be attributed to the presence of maize bran in several samples of complete feed from farmers. Studies have shown that aflatoxin tends to colonize maize right from the field depending on environmental conditions (temperature& humidity) as compared to other grains [23].

The relatively lower aflatoxin contamination (30%) among processors could be attributed to the sorting of ingredients to eliminate foreign materials that would damage the milling machine. Ingredients with visible contamination were disposed of in the process.

The factories revealed that 100% of samples analyzed were within acceptable aflatoxin contamination levels (20 ppb). Results from Croatia revealed 38.1% of maize feed ingredients from factories to be positive for aflatoxins with 28.8% of the samples contaminated beyond acceptable levels (20 ppb) [22]. The results from the current study may be possible to the quality assurance and availability of personnel, materials, tools, and equipment like silos for the storage of ingredients for a longer period. The aflatoxin contamination among traders (60%) could be associated with a longer storage period for the huge amounts of ingredients bought at cheaper prices with hopes of high sales during scarcity evidenced by the records accessed during the study.

The overall peroxide value along the fish feed and ingredients value chain in the current study indicated that 64.4% of samples were "above acceptable" peroxide value (20 meq/kg). The overall average P.V along the value chain was

27.70 meq/kg. The current study revealed no significant difference in peroxide value with value chain activity. A similar study to evaluate the quality of feed fats and oils in the U.S.A revealed a relatively lower P.V range of 0.4 - 7.3 meq/kg [12]. A study from Norway to determine lipid oxidation products in vegetable oils and marine omega-3 supplements reported a P.V range of 1.04 - 10.38 meq/kg & 0.60 - 5.33 meq/kg respectively [24].

The individual value chain activity with fish feeds and ingredients beyond acceptable P.V were 90%, P.V range 15.40 - 44.24 meq/kg among traders, 65%, P.V range 1.93 - 39.60 meq/kg among farmers, 50%, P.V range 14.51 - 30.20 meq/kg among processors and 30.40 - 39.80 meq/kg from the landing site. The 100% peroxidation of samples above the acceptable levels at landing sites in the current study could be attributed to the temporary storage areas for silverfish coupled with rains in March that extend days of drying. The high percentage of samples above acceptable P.V in traders (90%) than processors (50%) could be attributed to the milling, re-packaging of fish feeds and ingredients in whole bags, and the use of additives (premix) that contain anti-oxidants.

Anisidine value in the current study revealed that 64.4% of the fish feeds & ingredients that were beyond acceptable peroxide values (20 meq/kg) were above acceptable AnV (20). The overall average Anisidine value was (19.30) as compared with a lower average AnV of 5.25 reported in the USA [25]. However, the current study revealed no significant differences in Anisidine value within value chain activity. Ardo [25] attributed the low Anisidine value to the fact that the compounds that affect the AnV are secondary, but not terminal products of lipid peroxidation. He further noted that secondary compounds could be broken down to lower molecular weight compounds that are more volatile and leave the sample.

With the individual value chain activity, the current study for anisidine value revealed that 50% of the samples from the landing site were beyond acceptable levels, 46.15% in farmers, 33.3% in traders & 20% in processors. The relatively high percentage of Anisidine value above acceptable levels at the landing sites could be attributed to high levels of highly unsaturated (poly-unsaturated and mono-unsaturated) fatty acids which make it particularly susceptible to oxidative deterioration [16]. Lack of adequate storage facilities, as well as whole ingredient exposure to adverse conditions, can catalyze the process from primary to secondary rancidity. The relatively lower AnV above the acceptable level among farmers, traders, and processors in this study could be attributed to the presence of natural antioxidants such as tocopherols (vitamin E), carotenoids, and β–carotene in feeds and ingredients of plant origin [26].

The study revealed rodents & vermin and temperature (OR = 2.4, CI: 0.24 - 24.55 and OR = 1.8, 95% CI: 0.35 - 9.66 respectively), to be positively associated with "above acceptable" aflatoxin levels at storage areas for locally processed commercial fish feeds and ingredients at storage areas. There was no statistically significant difference in all the factors with aflatoxin contamination. The current study agrees with significant results from Benin that reported insect damage and temporary storage structures to be associated with increased aflatoxin contamination but disagrees with the current study on storage period to be statistically significant with aflatoxin contamination [27]. The association between temperature and above acceptable aflatoxin level in the current study could be attributed to high moisture and temperature in the tropics which are favorable for fungal developments [28]. Furthermore, stress from high temperatures and damage by insects before harvest have been linked to contamination of crops (the major component of feeds) even before reaching the storage areas [29].

Fish Feeds and ingredients with above acceptable storage period were more likely to be "above acceptable" peroxide value (20 meq/kg). The current study revealed no statistically significant difference in storage factors and peroxide value. The results of the current study disagree with the results on the storage stability of value-added products from sunflower kernels that revealed the type of packaging material to be statistically associated with high peroxide value [30]. The results in the current study could be attributed to the absence of regulations and an authorized body to monitor the trade and processing of local fish feeds that contain unstable high lipid ingredients mostly evidenced among traders.

Feeds and ingredients with not acceptable storage temperatures were more likely to test with an "above acceptable" anisidine value (>20). The study revealed no statistical difference in storage factors with anisidine value. A study with significant results revealed the rate of oxidation to be strongly dependent on storage temperature with an optimum temperature of 22˚C [13]. The results in the current study could be attributed to acceleration of the oxidation reaction by elevated temperature to both initiate and catalyze the production of free radicals that react with unsaturated oils and fats with omnipresent oxygen in storage areas [31].

5. Conclusions

The study revealed that 100% of fish feeds and ingredients along the value chain are positive for aflatoxin contamination. Fifty-one percent (51%) of the positive samples were "above acceptable" aflatoxin levels (20ppb). "Above acceptable" aflatoxin levels were more evident in farmers (65%) and not evidenced in factories.

All the fish feeds and ingredients were positive for peroxide value. The overall "above acceptable" peroxide value (20 meq/kg) along the value chain was 66.6%. Within the value chain, above acceptable peroxide values were more evident at the landing site and not evident in factories. For the Anisidine value, 100% of the "above acceptable" peroxide value (n = 29) was positive. Only 37.9% were "above acceptable" Anisidine value (20). Anisidine above acceptable level was more evident at landing sites.

The current study revealed storage factors of temperature and rodents & vermin to be more associated with aflatoxin contamination. Storage period, sunlight, and temperature respectively to have more impact on peroxide value and temperature were more associated with the above acceptable Anisidine value in locally processed commercial fish feeds and ingredients though no factors were statistically significant at multivariate logistic regression.

There is high potential for the use of locally processed commercial fish feeds and ingredients in the Kampala and Wakiso districts but requires producers to source the feeding materials from factories. There is a big need to sensitize and train farmers on how to maintain and improve the quality of locally processed commercial fish feeds and ingredients. There is a need to fast track enactment of the Animal Feed Law to provide more legal and technical guidance on the local processing and handling of fish feeds along with the nodes of the value chain in Uganda. Further research, intensive incubation, and field evaluations are needed to develop the capacity of local producers toward fish feed production with densified products like pellets.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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