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High performance liquid chromatography assessment of antibiotic residues in poultry and fish feeds in Bangladesh

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ABSTRACT

The extensive use of antibiotics in animal feed in Bangladesh raises concerns in commercial poultry and fish feeds, posing risks of antibiotic resistance in the food chain. This study aimed to critically examine feed safety and quality by assessing tetracyclines (oxytetracycline, tetracycline, and chlortetracycline) and amoxicillin in commercially produced poultry and fish feeds. Fifteen feed samples from different areas in Dhaka and Gazipur were collected and subjected to extraction and analysis with a high-performance liquid chromatography (HPLC) with photodiode array detection (PDA) method. The HPLC method was validated for linearity (R^2>0.999), accuracy, and precision, limit of detection or LOD (0.72 to 1.77 μgkg^{-1}) and limit of quantification or LOQ (1.77 to 3.69 μgkg^{-1}), meeting European Union and Codex Alimentarius Commission standards. Matrix-matched calibration curves for each antibiotic in poultry and fish feeds exhibited excellent linearity. The sensitivity of the HPLC system was demonstrated through low LOD and LOQ. Results indicated moisture content 6.58-11.22% in poultry feeds and 6.58-11.02% in fish feeds, while ash content 4.42-12.83% in poultry feeds and 4.94-8.23% in fish feeds. Antibiotics were found to be below detection limits in all feed samples, suggesting their absence or levels below the maximum residue limits established by regulatory bodies. The study highlights the importance of monitoring antibiotics in feed to ensure food safety and mitigate antibiotic resistance risks. Further research on a larger scale is recommended to validate these findings and contribute to the development of robust regulatory frameworks for antibiotic use in animal feed production in Bangladesh.

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1. Introduction

In Bangladesh, there is a concern around the wide spread use of antibiotics in food production from animals and the threat of transmitting antibiotic resistance (Ghosh et al., 2007) within the food chain (Laxminarayan et al., 2013; Adebowale et al., 2016) and other routes. In Bangladesh, the majority of commercial chicken farms use antibiotics during production, primarily for prophylactic purposes, according to a cross-sectional study (Imam et al., 2020). Another study found similar patterns in broiler farms, with all poultry farms using antibiotics and a significant portion utilizing them for prophylaxis (Islam et al., 2016). To address this issue, the Bangladesh government implemented the "Bangladesh Fish Feed and Animal Feed Act 2010," which bans the incorporation of antibiotics, growth hormones, steroids, and insecticides in animal feed manufacturing (Chowdhury et al., 2022; Fish Feed and Animal Feed Act, 2010). Broad spectrum antibiotics such as oxytetracycline (OTC), tetracycline (TC) (Mehtabuddin et al., 2012), chlortetracycline (CTC), and amoxicillin (AMX) are used as an antidote (Molt, 2000) in livestock animals and poultry farms (Leekha et al., 2011) as well as for the treatment of bacterial infections. Bacterial resistances evolve when antibiotics are used and taken unnecessarily. In such situations, antibiotics may not perform effectively when residues from poultry meat and fish, are transferred to humans, posing a serious risk. The Fish and Animal Feed Act (Fish Feed and Animal Feed Act, 2010) in Bangladesh forbids the use of some antibiotics such as chloramphenicol (CAP) in feed. When farm fish and animals affected by bacterial infections, the common treatment is to use appropriate antibiotics to treat and prevent a catastrophic death rate in fish and animals. Several farms have demonstrated the effectiveness of this approach, and the World Health Organization (WHO) for animal health has endorsed these efforts to confirm their role in ensuring a stable protein supply for humans. But disadvantages arise gradually depending on the specific antibiotic use such as the loss of advantageous bacterial growth in the body (Gaskins et al., 2002) and continued to multiply in the presence of therapeutic levels of an antibiotic (Spellberg et al., 2013). The uses of antibiotics as growth promoters in feed additives for livestock farming (Barton et al., 2000, Rahman et al., 2022) benefit the farm owners. Furthermore, the minimal antibiotics dosages employed for promoting animal growth in livestock farming are efficacious (Conly et al., 2005) and they may suppress some infectious diseases such as anthrax, cholera etc (Merck, 2017; Chey et al., 2017).

In comparison to other methods for assessing antibiotics in poultry and fish feeds, HPLC/PDA offers several advantages. Unlike traditional methods like microbiological assays or immunoassays, HPLC/PDA provides superior sensitivity, specificity, and the ability to simultaneously analyze multiple antibiotics (Lotfipour et al.,

2010). Additionally, it enables the quantification of antibiotics at low concentrations with high accuracy. Compared to techniques such as liquid chromatography-mass spectrometry (LC-MS/MS), HPLC/PDA typically has lower initial setup costs and is more accessible in resource-limited settings, making it a practical choice for routine analysis of multiple antibiotics (Ali, 2022). In Bangladesh, many people are living on domestic production like cattle, goat and broiler chicken (Hinton et al., 2000) and to supply more animal proteins for better nutrition. But there is a lack of awareness regarding the residual effects of antibiotics that may lead to resistance due to continuous use (Mou et al., 2021). Therefore, the objectives of the present studies include determining the moisture and ash content, identifying the levels of three tetracyclines such as tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and amoxicillin (AMX) in poultry and fish feed samples commercially produced in Bangladesh using an effective high performance liquid chromatographic method. The study aims to provide valuable insights into the prevalence and distribution of antibiotics in animal feeds within the Bangladeshi, shedding light on potential risks to human health and the environment. By quantifying antibiotics, the study seeks to assess the adherence to regulatory guidelines and identify areas for improvement in farming practices and feed manufacturing processes. Ultimately, the objective is to contribute to evidence-based policymaking and regulatory interventions aimed at reducing antibiotic usage, mitigating the emergence of antibiotic resistance, and ensuring the safety and sustainability of poultry and fish farming in Bangladesh.

Table 1. Sample name, ID and area of collected feed samples

Sample ID	Local name	Sampling area	Sample ID	Local name	Sampling area
PF1	House feed	Ananda bazar	PF9	Boiler grower feed	Gazipur
PF2	Sonali grower feed	Ananda bazar	FF1	Saudi Bangla fish feed	Ananda bazar
PF3	Sonali starter feed	Ananda bazar	FF2	Sunny fish feed	Ananda bazar
PF4	Boiler starter feed	Ananda bazar	FF3	Shapla fish feed	Gazipur
PF5	Layer starter feed	Ananda bazar	FF4	Capital fish feed	Gazipur
PF6	Layer mash feed	Ananda bazar	FF5	Quality fish feed	Savar
PF7	Boiler feed	Gazipur	FF6	Bismillah fish feed	Savar
PF8	Boiler chick feed	Gazipur	-	-	-

2. Materials and methods

2.1. Sampling strategy

Fifteen (n=15) different types of poultry (PF_1-PF_9) and fish feed (FF_1-FF_6) samples (200 g of each) were collected from different areas of Dhaka and Gazipur city, Bangladesh (Table 1). Each feed sample was first wrapped with clean air tight zip-locked bags, label properly and brought to the laboratory of the department of chemistry, University of Dhaka. The samples were kept in a separate clean zone of the laboratory at room temperature until extraction.

2.2. Reagents and chemicals

Ethyl acetate, n-hexane, acetone, acetonitrile and methanol were used purchased from RCI-LABSCAN, EMerck-Germany, BDH-England, SMART-LAB and sulpeco, respectively. C-18 powder, oxalic acid and pure silica sand were purchased from Sigma-Aldrich, Germany. Deionized water was collected from the Mill-Q System (Denmark and USA).

2.3. Moisture and ash content

In the analysis of commercially available poultry and fish feed in Dhaka and Gazipur city, the assessment of moisture and ash content was meticulously conducted. These key parameters provide valuable insights into the quality and nutritional composition of the feeds, offering crucial information for both producers and consumers in the poultry and aquaculture industries. For the determination of moisture and ash content, 2.0 g of each feed samples was taken in separate pre-weighed dry crucible and kept in oven temperature at 105 °C and 700 °C for about 3 and 4 hours, respectively and final weight was recorded to calculate the percentage of moisture and ash content (Shahjahan et al., 2021).

2.4. Sample extraction and clean up

Homogenized poultry/fish feed (5 g) was taken in a 250 mL stoppered conical flask containing 30 mL n-hexane and left overnight at room temperature. Next, using solid phase extraction, the soluble part was discarded. Ethyl acetate (20 mL) was added to the remaining sample and the filtrate was evaporated completely. A mixture of hexane-water (20 mL 1:1) was added to the dried and the n-hexane part was discarded. Next, the aqueous part was partitioned with ethyl acetate (2 mL) and organic layer was dehydrated through cotton filter over anhydrous sodium sulfate before being evaporated completely. The residue was re-dissolved in ethyl acetate (2.0 mL). The ethyl acetate extract was applied to a preconditioned C-18 reversed phase silica gel column with 3 mL of acetonitrile. The final volume was adjusted to 2 mL by passing N_2 (Doyle, 2006; Ramatla, et al., 2017).

2.5. Preparation of stock and working standard solutions

The primary standard solutions (1000 mgL^{-1}) of TC, OTC and CTC were prepared separately by dissolving certified standards (99.99% purity) in MeOH while AMX was prepared with MeOH and deionized H_2O (1:1) mixture in 10 mL volumetric flasks. Then, a definite amount of the solution was taken for making calibration curve standards of concentration 125, 100, 75, 50, 25 and 10 μgL^{-1} for TC, OTC and CTC and 25, 20, 15, 10, 5 and 1 μgL^{-1} for AMX.

2.6. Instrumental conditions of HPLC

High-Performance Liquid Chromatography equipment with Photo Diode Array Detector (HPLC PDA Detector, Shimadzu, CTO10ASVP) and shim-pack GISS reversed phase C18 column (C18: 250 × 4.6 mm i.d. 5 μm) and rheodyne injector (20 μL samples loop) were used to carry out the analysis of target antibiotics. At first, the system was washed by passing MeOH and H_2O mixture in different proportions, then conditioned by passing mobile phase $C_2H_2O_4$, ACN and MeOH (70:20:10) mixture for OTC, TC and CTC, and sodium acetate and acetonitrile (70:30) mixture for AMX. Blank injection was done first to check whether the column was clean or not. All standards and samples were injected (20 μL) in isocratic mode. The run times for OTC, TC, CTC was 15 min and for AMX was 10 min. The retention times (RT) were found 4.10, 4.59, 8.32, and 3.37 min for OTC, TC, CTC and AMX, respectively, with an oven temperature of 40 °C (TCs) and 30 °C (AMX) and flow rate 1.0 $mLmin^{-1}$. All standards and samples (Fig. 1 and 2) were detected at 360 nm for OTC and TC, 375 nm for CTC and 230 nm for AMX. Lab-

Solution software was used for the instrumental control throughout the analysis.

2.7. Method validation parameters

The method was validated for linearity, accuracy, and precision in accordance by the EU Commission Decision, 2002/657/EC (European Communities, 2002). The standard deviation of the response (peak area) was utilized in the calculation, employing the linear equation obtained from the calibration curves. Limit of detection (LOD) and limit of quantification (LOQ) were determined (Mou et al., 2021) by considering the peak area of each standard as 3 and 10 times higher than the baseline noise, maintaining a signal-to-noise ratio of 3:1 and 10:1, respectively. Recovery was calculated from matrix matched calibration curve at 2 different spiking levels. The accuracy was evaluated by percentage recoveries (Mou et al., 2021). Repeatability (intra-day, n=5) and reproducibility (inter-day, n=15) of the method were found at 50 μgkg^{-1} and 75 μgkg^{-1} levels for TCs and 10 μgkg^{-1} and 15 μgkg^{-1} levels for AMX. The precision estimated by determining the co-efficient variations (Parvin et al., 2022). Calculating the matrix effect (Mou et al., 2021) involved a comparison with the calibration curve of standards prepared using both mobile phase and matrix matching matched samples.

Table 2. Moisture and ash content in the poultry and fish feed. Data of mean ± standard deviation were from the triplicate experiment.

Sample ID	Moisture content ± SD (%)	Ash content ± SD (%)	Sample ID	Moisture content ± SD (%)	Ash content ± SD (%)
PF_1	10.10 ± 0.48	5.04 ± 0.18	PF_9	7.45 ± 0.46	12.83 ± 0.39
PF_2	11.02 ± 0.35	4.42 ± 0.10	FF_1	6.58 ± 0.11	6.58 ± 0.12
PF_3	6.79 ± 0.13	6.79 ± 0.13	FF_2	9.43 ± 0.62	4.94 ± 0.25
PF_4	6.58 ± 0.11	6.58 ± 0.11	FF_3	11.02 ± 0.34	4.42 ± 0.11
PF_5	9.43 ± 0.62	4.94 ± 0.24	FF_4	9.24 ± 0.13	6.16 ± 0.02
PF_6	11.22 ± 0.86	5.26 ± 0.10	FF_5	6.78 ± 0.12	8.23 ± 0.13
PF_7	6.78 ± 0.13	8.23 ± 0.13	FF_6	10.10 ± 0.47	5.04 ± 0.15
PF_8	9.24 ± 0.14	6.16 ± 0.02	-	-	-

Table 3. Retention time, linearity, regression lines (y = mx + c) and regression coefficient (R^2)

Antibiotics	RT (min)	Linearity (μgkg^{-1})	LOD (μgkg^{-1})	LOQ (μgkg^{-1})	Matrix matched calibration curves (y = mx + c)			
					Poultry feed	R^2	Fish feed	R^2
OTC	4.10	10-125	1.15	3.45	y = 192.40x – 126.12	0.9991	y = 193.55x – 163.31	0.9990
TC	4.59	10-125	1.23	3.69	y = 189.30x + 839.58	0.9991	y = 187.32x + 842.93	0.9990
CTC	8.32	10-125	1.77	1.77	y = 120.65x – 547.81	0.9990	y = 118.24x – 470.25	0.9991
AMX	3.37	1-25	0.72	2.16	y=2074.50x +2262.70	0.9991	y = 2079.30x + 2294.50	0.9990

Table 4. Recoveries of TCs with relative standard deviation (RSD) in poultry and fish feed.

Day	Spiking level (μgkg^{-1})	Poultry feed			Fish feed		
		OTC	TC	CTC	OTC	TC	CTC
Intra-day-1 n=5	50	96 ± 4.89	101 ± 3.92	101 ± 6.02	100 ± 6.31	100 ± 1.62	102 ± 6.24
	75	99 ± 6.03	100 ± 3.55	99 ± 4.89	99 ± 1.02	99 ± 2.60	101 ± 4.97
Intra-day-2 n=5	50	99 ± 7.36	100 ± 2.43	103 ± 5.13	102 ± 4.19	99 ± 3.75	102 ± 5.44
	75	102 ± 4.68	99 ± 1.72	104 ± 4.47	100 ± 1.50	102 ± 2.35	102 ± 3.43
Intra-day-3 n=5	50	102 ± 5.57	99 ± 3.93	101 ± 5.48	100 ± 2.73	99 ± 2.09	102 ± 4.09
	75	101 ± 5.03	99 ± 2.63	103 ± 5.64	99 ± 1.03	98 ± 2.91	98 ± 4.96
Inter-day n=15	50	99 ± 5.94	100 ± 3.42	101 ± 5.54	100 ± 4.35	99 ± 2.49	102 ± 5.26
	75	101 ± 5.25	97 ± 2.63	102 ± 5.00	99 ± 1.18	100 ± 2.62	100 ± 4.45

3. Results and discussion

3.1. Moisture and ash content

The results of moisture and ash content analysis in poultry and fish feed samples are presented in (Table 2). In poultry feeds (PF_1-PF_9), the moisture content ranged from 6.58-11.22%, with PF_6 exhibiting the highest moisture content of 11.22 ± 0.86%. The ash content in poultry feeds varied from 4.42-12.83%, with PF_9 having the highest ash content of 12.83 ± 0.39%. In fish feeds (FF_1-FF_6), moisture content ranged from 6.58-11.02%, with FF_3 showing the highest moisture content of 11.02 ± 0.34%. The ash content in fish feeds varied from 4.42-8.23%, with FF_5 having the highest ash content of 8.23 ± 0.13%.

As per Magan and Lacey (1988), poultry and fish feed are considered safely stored with a moisture content of ≤15%. However, mold growth has been observed within the temperature range of 10-40 °C and at relative humidity exceeding 70% (Magan and Lacey, 1988). According to Bangladesh Standards and Testing Institute (BSTI), the ash content in the poultry and fish feed 20-35%. All the targeted feed samples analyzed in this study are comply with the above guideline values, although there are variable amount of moisture and ash content in the samples. These findings indicate notable variability in both moisture and ash content among the different poultry and fish feed samples. The observed differences could be attributed to variations in feed composition and processing methods. Understanding moisture and ash content is crucial for assessing the nutritional quality and stability of the feeds, providing valuable information for producers and consumers in the poultry and aquaculture industries (Hossain et al., 2023). Further research and monitoring are essential to establish baseline data and ensure consistency in feed quality for sustainable animal farming practices.

3.2. Method validation

HPLC/PDA method employed for the analysis of antibiotics in poultry and fish feeds underwent comprehensive validation, ensuring its accuracy and reliability. Table 3 presents the key validation parameters for OTC, TC, CTC, and AMX. RT values ranged from 4.10 to 8.32 min, confirming the distinct identification of each antibiotic peak. The linearity of the matrix-matched

calibration curves was excellent, with regression coefficients (R^2) exceeding 0.999 in both poultry and fish feeds for all antibiotics. LOD ranging from 0.72 to 1.77 μgkg^{-1} underscored the method's sensitivity to trace amounts of antibiotics. Similarly, LOQ ranging from 1.77 to 3.69 μgkg^{-1} demonstrated the method's ability to accurately quantify antibiotic in feeds.

The established regression lines (y = mx + c) for each antibiotic exhibited consistent slopes and intercepts in both poultry and fish feeds, further affirming the method's robustness. These validation results collectively support the suitability and precision of the HPLC method for the routine analysis of antibiotics in poultry and fish feeds, contributing to the overall goal of ensuring food safety and mitigating antibiotic resistance risks in the food chain. The HPLC method's robustness and reliability were further substantiated through a thorough validation of recoveries and precision (Table 4 and 5) for TCs and AMX in poultry and fish feeds. For TCs, the intra-day recoveries at spiking levels of 50 μgkg^{-1} and 75 μgkg^{-1} consistently ranged between 96% and 104% in poultry feed and 98% to 102% in fish feed. The relative standard deviations (RSD) for these recoveries were 1-7% within the acceptable range of ≤20% (CODEX Alimentarius, 2017). Similarly, inter-day recoveries at the same spiking levels showed consistent results, emphasizing the method's repeatability and reliability. In the case of AMX, the intra-day recoveries at spiking levels of 10 μgkg^{-1} and 15 μgkg^{-1} were consistently around 99%, with RSD values 1-4% within the acceptable limit of ≤20% (CODEX Alimentarius, 2017). The inter-day recoveries demonstrated similar trends, affirming the method's accuracy and precision for AMX in both poultry and fish feeds.

These results indicate that the HPLC method provides reliable and reproducible measurements of antibiotics in feeds, meeting the stringent criteria set by regulatory standards (ICH, 2022). The low RSD values and high recovery percentages underscore the precision and accuracy of the method, supporting its suitability for routine analysis and monitoring of antibiotics in poultry and fish feeds, contributing to the assurance of food safety.

Table 5. Recoveries of AMX with relative standard deviation (RSD) in poultry and fish feed

Day	Spiking level (μgkg^{-1})	Poultry feed	Fish feed
Intra-day-1 n=5	10	98 ± 4.62	99 ± 2.84
	15	99 ± 2.44	98 ± 2.58
Intra-day-2 n=5	10	100 ± 3.16	98 ± 3.30
	15	99 ± 1.10	99 ± 2.83
Intra-day-3 n=5	10	99 ± 3.51	98 ± 3.57
	15	99 ± 2.97	100 ± 1.63
Inter-day n=15	10	98 ± 3.76	99 ± 3.24
	15	98 ± 2.17	99 ± 2.35

3.3. Matrix effects

The matrix effect assessment for TCs in poultry and fish feeds, as illustrated in (Table 6), reveals the impact of the matrix on the HPLC method's accuracy and sensitivity.

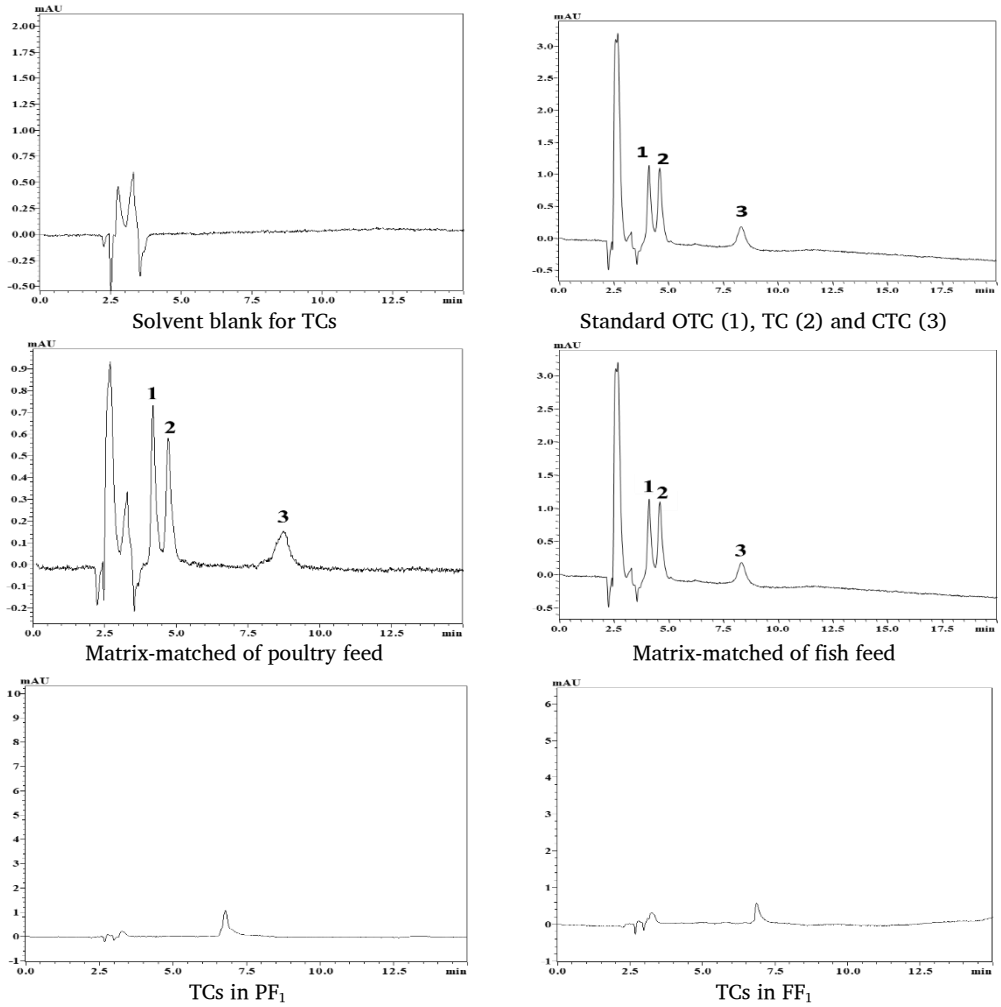

Fig. 1. Chromatograms of solvent blank, matrix-matched and some real samples for TCs

For TCs and AMX in poultry feed, at concentrations ranging from 10 to 125 µgkg^{-1} and 1-25 µgkg^{-1}, the matrix effect percentages varied from 97-107% for OTC, 99-106% for TC, 99-107% for CTC and 92-106% for AMX. Similarly, in fish feed, the matrix effect ranged from 97-104% for OTC, 98-106% for TC, 96-106% for CTC, and 93-105% for AMX.

These results indicate that the matrix-matched calibration curves effectively compensated for matrix interferences, ensuring accurate quantification of TCs in both poultry and fish feeds. The mild matrix enhancement effect within the specified ranges supports the reliability of the HPLC method. The values obtained underscore the method's robustness and suitability for routine analysis, emphasizing its potential for accurately determining antibiotics in diverse feed matrices. These findings contribute to the establishment of a dependable analytical framework for monitoring antibiotics in animal feeds, essential for safeguarding food safety and mitigating antibiotic resistance risks.

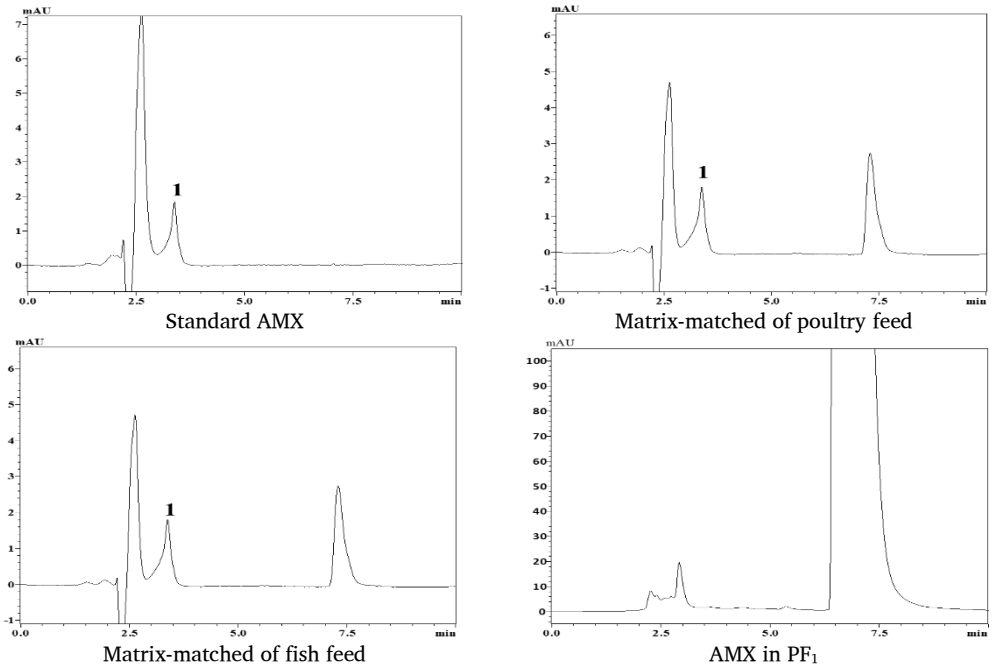

Fig. 2. Chromatograms of matrix-matched and a real sample for AMX

Table 6. Matrix effect for the TCs and AMX in poultry and fish feed

Concentration (µgkg^{-1}) of TCs	Poultry feed (%)			Fish feed (%)			Concentration (µgkg^{-1}) of AMX	Poultry feed (%)	Fish feed (%)
	OTC	TC	CTC	OTC	TC	CTC			
10	105	99	107	97	104	104	1	106	105
25	102	103	107	103	103	96	5	92	93
50	99	102	99	104	98	102	10	102	103
75	104	106	103	103	106	105	15	97	98
100	107	106	106	101	104	106	20	103	103
125	97	105	103	99	104	99	25	101	101

Table 7. Amount (µgkg-1) of antibiotics detected in the poultry and fish feed

Sample ID	OTC	TC	CTC	AMX	Sample ID	OTC	TC	CTC	AMX
PF_1	BDL	BDL	BDL	BDL	PF_9	BDL	BDL	BDL	BDL
PF_2	BDL	BDL	BDL	BDL	FF_1	BDL	BDL	BDL	BDL
PF_3	BDL	BDL	BDL	BDL	FF_2	BDL	BDL	BDL	BDL
PF_4	BDL	BDL	BDL	BDL	FF_3	BDL	BDL	BDL	BDL
PF_5	BDL	BDL	BDL	BDL	FF_4	BDL	BDL	BDL	BDL
PF_6	BDL	BDL	BDL	BDL	FF_5	BDL	BDL	BDL	BDL
PF_7	BDL	BDL	BDL	BDL	FF_6	BDL	BDL	BDL	BDL
PF_8	BDL	BDL	BDL	BDL	-	-	-	-	-

Bangladesh Food Safety Authorities (BFSA), European Union (EU) and Codex Alimentarius Commission sets maximum residue limit (MRL) for TCs and AMX are 100 (BFSA, 2013; FAO, 2004) and 10 µgkg^{-1} (Irum et al., 2014) in poultry and fish feed. In this study, a meticulous analysis using HPLC methods on fifteen commercial poultry and fish feed samples in Bangladesh were done. The results revealed that all targeted antibiotics, including OTC, TC, CTC, and AMX, were found below the detection limit (BDL). This indicates that the concentrations of these antibiotics were either absent or present at levels lower than the method's detection capabilities (Table 7). On the other hand, Sani et al., (2023) analyzed 120 feed samples from Jamalpur, Mymensingh Netrokona and Sherpur which are near from Dhaka city. They found 0.50-9, 85, 1.56-150.21 5.80, 37.43- 77.08 µgkg^{-1} of doxycycline, OTC and ciprofloxacin, AMX, respectively. This indicates the use or misuse trend of antibiotics in poultry feed using poultry owner unconsciously although most of them are in below MRL values (Sani et al., 2023). The absence or minimal presence of antibiotics in the analyzed feed samples underscores the adherence to regulatory standards and emphasizes the safety and quality of the commercial poultry and

fish feeds in Bangladesh. These findings contribute valuable insights into the overall feed safety, supporting the importance of stringent monitoring practices in ensuring the production of safe and high-quality animal feeds.

4. Conclusion

In summary, the assessment of antibiotics in commercial poultry and fish feeds in Bangladesh using HPLC/PDA was comprehensively studied. The moisture and ash content were determined, the HPLC method was validated, and the recoveries and matrix effects for TCs and AMX were evaluated. Significant variability in moisture and ash content among feed samples provided insights into their quality. The validated HPLC method demonstrated excellent linearity and sensitivity, including its reliability to international standards. Consistent and accurate recoveries, along with successful matrix compensation, affirmed the method's precision. Minimal antibiotic residues, below regulatory limits, underscore the importance of monitoring for food safety and combating antibiotic resistance. These findings contribute to a robust analytical framework for ongoing monitoring and regulatory support in Bangladesh's animal feed industry.

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Conflict of interest

The authors declare there is no conflict of interest in this research.

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