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Comparison of the Efficiency of Some Non-Thermal Techniques in Detoxifying Aflatoxin B1, Ochratoxin A, and Fumonisin B1 from Feeds of Poultry

Hiba S Alnaemi*[1], Tamara N Dawood[2], Qais Algwari[3]

[1]Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq,
[2]Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq,
[3]Department of Electronics, College of Electronic Engineering, Nineveh University, Mosul, Iraq

***Correspondence**:
hibaalnaemi6@uomosul.edu.iq
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A B S T R A C T

Atmospheric pressure corona discharge (APCD), ultraviolet C with ozone (UVC w/ O_3), and plasma treated water (PTW) have lately been spotlighted in the food industry as non-thermal techniques (NTTs) for detoxifying mycotoxins, due to their unique features. The efficiency of these techniques in detoxifying aflatoxin B1 (Afla B1), ochratoxin A (Ochra A), and fumonisin B1 (Fum B1) in feeds of poultry and their effects on quality of feed were inspected. Samples of feed were subjected to APCD, UVC w/ O_3, and PTW for 10, 20, and 60 min. Mycotoxin concentrations were determined by competitive enzyme-linked immunosorbent assay (ELISA), and outcomes were verified by high-performance liquid chromatography (HPLC). Standard analytical methods were adopted for analyzing feed components and determining peroxide values (PVs). Subjecting samples to APCD, UVC w/ O_3, and PTW for 10 min resulted in degradation of Afla B1 to levels of 46.6, 38.9, and 28.9%, Ochra A to 49.8, 35.9, and 29.9%, and Fum B1 to 58.6, 42.6, and 35.9%, respectively, for 20 min to Afla B1 levels of 57.7, 46.6, and 32.9%, Ochra A 68.9, 45.3, and 38.5%, and Fum B1 75.7, 49.9, and 41.6%, respectively, for 60 min to Afla B1 levels of 83.2, 65.7, and 33.8%, Ochra A 84.2, 73.6, and 40.7%, and Fum B1 84.8, 71.2, and 43.4%, respectively. The main conclusion of the study is that APCD and UVC w/ O_3 can be adopted to efficiently degrade Afla B1, Ochra A, and Fum B1 in feed while maintaining its quality. According to their impact on feed quality, techniques can be ranked as follows: APCD > UVC w/ O_3 > PTW.

Keywords: aflatoxin B1, fumonisin B1, ochratoxin A, poultry feed, non-thermal technique

INTRODUCTION

Mycotoxins, toxic compounds created as secondary metabolites by mycotoxigenic molds, have a diversified of toxic influences on humans and animals (**1-3**). Mycotoxin-producing molds are widely disseminated throughout the world, resulting in mycotoxin emergence in the food series (**4-6**). Individuals can be subjected to mycotoxins directly and indirectly through eating contaminated foods of plant and animal origin, respectively (**1,7**). A wide variety of mycotoxins have been recognized, but the constantly faced mycotoxins that comprise a disquiet for the health of animals and humans and are of economic prominence are aflatoxin B1 (Afla B1), ochratoxin A (Ochra A), and fumonisin B1 (Fum B1). Multiple pathological influences can arise from exposure to these mycotoxins in the food chain as immunotoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, carcinogenicity, mutagenicity, and teratogenicity (**1,8-11**).

Since mycotoxins comprise a significant health intimidation to animals and humans and cause huge economic casualties, finding ways to eliminate or disrupt mycotoxins in feeds and foods is urgent, and these ways are broadly classified into physical, chemical, and biological methods (**12-19**). In recent decades, non-thermal physical techniques (NTTs) have emerged, as they require a small budget, minimal time exhaustion, minimal side effectiveness on the food matrix, and their potential application to whole sorts of feeds and foods (**20**). Atmospheric pressure corona discharge (APCD) in air, a type of NTTs, is one of the simplest cold plasma (CP) jets and is recognized for its ability to produce powerful oxidants, namely reactive oxygen and nitrogen species (RONS) (**21**). These RONS have the greatest influence in mycotoxin degradation by rapidly reacting with specific multiple bonds, various active rings, and efficacious combinations attached to the construction of mycotoxins resulting in less toxic or non-toxic components than the authentic component (**22**). The efficacy of APCD in degrading mycotoxins in feed has not been notarized, although corona discharge (CD) for Afla B1 destruction has only been investigated in one study on slides and in artificially contaminated cereal crops (**21**). Treating water by discharging plasma, another NTT, also produces a multitude of RONS as a result of a series of complicated processes that occur during treating of water by plasma (**23**). The properties of the resulting plasma-treated water (PTW) rely on the chemical interactions among reactive species (RS) created by the CP and those occurring in the water, which modify the pH, electrical conductivity (EC), and oxidation-reduction potential (ORP) of the resulting water (**24**). Secondary RS created in water treated by plasma plays a significant role in destroying mycotoxins (**25**). With the recent emergence of PTW technique, no studies have been conducted on Afla B1, Ochra A, and Fum B1 detoxification from foods and feeds utilizing this technique, with the exception of the study that indicated the effectiveness of PTW with CP in degrading Afla B1 (**26**). Ultraviolet (UV) radiation is an efficacious NTT employed to destroy chemical contaminants, such as mycotoxins, through photolysis (**27**). UVA (315–400 nm), UVB (280–315 nm), UVC (200–280 nm), and vacuum ultraviolet (VUV) (100–200 nm) are the main sorts of UV radiation. The UV spectrum has adequate energy to destroy the chemical bonds within mycotoxins, causing their destruction and detoxification (**28**). An ozone-generating UV lamp can deliver UV light at two peaks in the UV spectrum, 254 nm (UVC) and 185 nm (VUV), referred to as "germicidal" and "ozone producing", respectively. The VUV light emitted from this lamp interacts with oxygen to break it down into oxygen atoms, which are extremely unstable atoms that combine with oxygen to form ozone, a vigorous oxidizer (**29**). The effectiveness of low-pressure UVC lamps that generate ozone in degrading mycotoxins has not yet been studied. Studies investigating the synergistic effect of UVC with O_3 on mycotoxin degradation have been scarce, with only one study reporting its effect on aflatoxin degradation utilizing an ozone-generating dielectric barrier discharge and a UVC lamp (**30**). Although these three techniques (APCD, UVC w/ O_3, and PTW) share the characteristic of demonstrating mycotoxin destruction effects through non-thermal mechanisms by producing numerous potent ROS, as they all belong to the family of advanced oxidative processes, they vary in their main mode of operation, the specified RS they generate, and their most convenient applications. Thus, selecting the most appropriate technique in terms of its efficiency in degrading mycotoxins in foods and feeds demands further investigation (**31**).

Comparison of APCD, UVC w/ O_3, and PTW in terms of their efficiency in detoxifying mycotoxins from feed with their effect on feed quality has not been documented. Therefore, the study was designed to compare the effectiveness of these NTTs in detoxifying Afla B1, Ochra A, and Fum B1 from feed of poultry, and to compare their impact on feed quality.

MATERIALS AND METHODS

Samples of Feed

Samples of feed (60 samples) were collected from feed mills and poultry ranches in Ninevah Province, northwest Iraq, during the interval from April to September 2022. Representative 1 kg samples were gained, milled, and sieved for mycotoxin detection. In order to acquire precise and reliable results, samples that exhibited existence of Afla B1, Ochra A, and Fum B1 in largest amounts (3 out of 53 positive samples) were thoroughly mixed and homogenized to gain a sample that was employed for treatment with APCD, UVC w/ O_3, and PTW.

Sample Processing Using Non-Thermal Techniques

The APCD system (Home-made by author No.3) composed of several components, of which the high-voltage provenance as an essential fraction. The APCD system operates in ambient air at atmospheric pressure with a supply voltage of 15 kV and a frequency of 100 Hz to create a discharge current of approximately 1 mA. The generation and quantification of RONS were performed according to the steps established in our previous publication (**32**). Feed samples (10 g) naturally contaminated with Afla B1, Ochra A, and Fum B1 were positioned in a modulated diaphanous polypropylene box (21.5×18×4 cm) in a tender layer of around 0.1 cm thickness and subjected to APCD for 10, 20, and 60 min at a distance of 1.5 cm between the electrode and the sample plate, with stirring every 5 min. The samples were examined in triplicate for each time period.

Plasma treated water was produced by APCD as stated by (**33**). The plasma was generated over the deionized water (diH_2O) surface at an electrode tip to water surface distance of approximately 1.5 cm. One hundred milliliters of diH_2O were positioned in a modified polypropylene vessel (8.3×8.3×8.3 cm) with a stainless-steel bottom to allow APCD creation with stirring each 5 min. APCD-treated water for 60 min (PTW-60) was instantly utilized to treat the samples. The attributes of treated water with respect to temperature, pH, EC, and ORP were clarified in detail by (**33**). The milled feed sample (30 g) was placed in a glass-

stoppered flask, mixed with PTW-60 (1:2.5 w/v), and shaken employing an orbital shaker (Benchmark Scientific, USA) for 10, 20, and 60 min. The control group was processed in the same manner with diH_2O. The samples were promptly desiccated at 40°C in an oven (Memmert, Germany) till they almost returned to the original moisture (9.85%). Moisture in feeds was measured based on AOAC method 930.15 (**34**). The samples were tested instantly after treatment in triplicate.

Generation of UVC w/ O_3 was achieved in a closed chamber (65×40×115 cm) equipped with a 38 W ozone-generating UVC lamp (360° UVC sterilization lamp, China) at a controlled room temperature of 25°C, without exterior lighting (**35**). The lamp contains two high-quality UVC tubes that produce UV light at two peaks (254 nm and 185 nm), where ozone is generated from the short wavelengths. To ensure safety, the lamp was equipped with a ten-second delay remote control. The sample (30 g) was evenly distributed on a 20 cm diameter tray in a thin layer (around 0.1 cm thick). The tray was put on the chamber shelf positioned 15 cm vertically under the UVC lamp for exposure periods of 10, 20, and 60 min. All experiments were carried out in triplicate. A digital UVC meter (RGM-UVC meter, China) was utilized to calculate the intensity of UVC radiation. To warm up the lamp, a period of 10 min was given before use. The calculated UVC intensity at a distance of 15 cm from the lamp was 0.963 mW/cm^2. The UVC dose was measured employing the equation: D = I*t, where D: UVC dose (mJ/cm^2), I: UVC radiation intensity (mW/cm^2), and t: time (sec).

The UVC doses at the three investigated time intervals (10, 20, and 60 min) were 577.8, 1155.6, and 3466.8 mJ/cm2, respectively. The O_3 concentration was continuously monitored and controlled using a calibrated ozone sensor (JXCT, China), as well as an Arduino-controlled system that used a feed-back loop to maintain the O_3 concentration at 10 ppm in real-time. When the measured concentration exceeded the fixed point, an exhaust fan would turn on through a relay module and vent the excess ozone out of the chamber; once the concentration got back to 10 ppm, the fan would turn off. The result of this closed-loop control was that the O_3 concentration was maintained at 10 ppm during the entire UVC/O_3 treatment process. A circulation fan was employed to ensure efficient ozone circulation. To check O_3 concentration, a portable O_3 gas detector (Shenzhen, China) was used every 10 min.

Determination of Mycotoxins in Feeds Using ELISA

The levels of target mycotoxins in feed samples pre- and post-nonthermal treatments were specified by ELISA kits (Elabscience Biotechnology Inc., USA). Sample initial preparations along with competitive ELISA procedures were accomplished as stated by the kit producer. An ELISA reader (HumaReader HS, Germany) was employed for optical density appreciation.

Affirmation of Mycotoxin Findings by HPLC assay

Highly performance liquid chromatography assay was accomplished on the samples before and after non-thermal treatments for the longest exposure period (60 min). The assay was executed for Afla B1 as described in (36), Ochra A as described in (**37**), and Fum B1 as described in (38). Standards of the studied mycotoxins were acquired from Sigma-Aldrich (USA). Purification of specimens were carried out employing SEP-PAK® silica cartridges and C18 cartridges (Waters Corporation, USA) for Afla B1 and Ochra A, respectively, and strong anion extraction (SAX) cartridges (InertSep SAX, GL Sciences, Japan) for Fum B1. Mycotoxins were determined employing HPLC with a fluorescent detector (Shimadzu Corp., Japan).

Levels of Mycotoxin Degradation

The degradation levels of target mycotoxins after subjecting to APCD, UVC w/ O_3, and PTW were calculated as follows: Mycotoxin degradation (%) = $(1-C_t/C_0) \times 100$, where C_t: mycotoxin concentration at time (t), and C_0: mycotoxin initial concentration at zero time.

Nutritional Components and Peroxide Values

The potential impact of non-thermal treatments on nutritional components and lipid oxidation in feed was evaluated by determining ash, crude protein, total carbohydrate, crude fat, and the peroxide value (PV) of the extracted fat. Ash content was determined following the method of Thiex et al. (**39**). Crude protein was estimated using the Kjeldahl procedure (**40**). Total carbohydrates were assessed according to the referenced method (**41**). Crude fat was determined using a Soxhlet extraction apparatus (Medilab, India) as described in (**42**). The peroxide value of the extracted fat was determined as described in (**43**).

Statistical Analysis

Statistical analysis of the data was achieved employing the One-Way Analysis of Variance of the Sigma Stat Version 3.10 (**44**). Means were compared using Duncan's Multiple Range Test at $P < 0.05$ (**45**).

RESULTS

The results of the efficiency of NTTs (APCD, UVC w/ O_3, and PTW) in the degradation of target mycotoxins from feed illustrated significant differences ($P < 0.05$) among them in the levels of mycotoxins degradation. Exposure of feed samples to APCD, UVC w/ O_3, and PTW for 10 min resulted in degradation of Afla B1 to levels of 46.6, 38.9, and 28.9%, Ochra A to 49.8, 35.9, and 29.9%, and Fum B1 to 58.6, 42.6, and 35.9%, respectively. Corona discharge under atmospheric pressure showed the highest levels of Afla B1 and Fum B1 degradation, followed by ultraviolet C with ozone, whereas plasma treated water showed the lowest levels of degradation. For Ochra A, identical findings were presented, although there was no significant difference ($P < 0.05$) between ultraviolet C with ozone and plasma treated water in Ochra A degradation levels (**Table 1**).

Results of exposing feed samples to APCD, UVC w/ O_3, and PTW techniques for 20 min demonstrated that Afla B1 degradation levels reached values of 57.7, 46.6, and 32.9%, Ochra A 68.9, 45.3, and 38.5%, and Fum B1 75.7, 49.9, and 41.6%, respectively. APCD also ranked first in terms of its degradation levels, while UVC w/ O_3 ranked second, and PTW ranked last (**Table 1**).

The degradation levels of target mycotoxins in samples subjected to 60-min APCD, UVC w/ O_3, and PTW techniques were registered as 83.2, 65.7, and 33.8% regarding Afla B1, 84.2, 73.6, and 40.7% regarding Ochra A, and 84.8, 71.2, and 43.4% regarding Fum B1, respectively. Mycotoxins were most efficiently degraded employing APCD, followed by UVC w/ O_3, whilst PTW displayed minimal level of degradation (**Table 1**).

Table 1. ELISA test results comparing non-thermal techniques for their efficiency in degrading Afla B1, Ochra A, and Fum B1 from poultry feeds after 10, 20, and 60 min of exposure

		Afla B1		Ochra A		Fum B1	
Exposure time (min)	**Type of exposure**	**C (µg/kg)**	**D (%)**	**C (µg/kg)**	**D (%)**	**C (µg/kg)**	**D (%)**
10	Control	23.7	0.00±0.00 ^{d}	164.7	0.00±0.00 ^{c}	6850	0.00±0.00 ^{d}
	APCD	12.6	46.6±3.12 ^{a}	82.2	49.8±2.70 ^{a}	2834	58.6±0.10 ^{a}
	UVC w/ O_3	14.5	38.9±1.00 ^{b}	105	35.9±3.23 ^{b}	3932	42.6±0.10 ^{b}
	PTW	16.8	28.9±1.39 ^{c}	114.9	29.9±3.07 ^{b}	4394	35.9±0.16 ^{c}
20	Control	23.7	0.00±0.00 ^{d}	164.7	0.00±0.00 ^{d}	6850	0.00±0.00 ^{d}
	APCD	10.0	57.7±1.00 ^{a}	50.9	68.9±1.87 ^{a}	1668	75.7±0.05 ^{a}
	UVC w/ O_3	12.7	46.6±0.23 ^{b}	89.9	45.3±1.77 ^{b}	3425	49.9±0.10 ^{b}
	PTW	15.9	32.9±1.76 ^{c}	100.8	38.5±2.85 ^{c}	4003	41.6±0.20 ^{c}
60	Control	23.7	0.00±0.00 ^{d}	164.7	0.00±0.00 ^{d}	6850	0.00±0.00 ^{d}
	APCD	3.97	83.2±0.08 ^{a}	25.9	84.2±0.68 ^{a}	1044	84.8±0.03 ^{a}
	UVC w/ O_3	8.09	65.7±2.93 ^{b}	43.3	73.6±1.46 ^{b}	1973	71.2±0.05 ^{b}
	PTW	15.7	33.8±1.53 ^{c}	97.3	40.7±2.42 ^{c}	3879	43.4±0.42 ^{c}

APCD: atmospheric pressure corona discharge, UVC w/ O_3: ultraviolet C with ozone, PTW: plasma-treated water, Control: without exposure. C: Concentration, D: Degradation represented as mean ± SEM (n=3). Vertical superscript letters that are not identical differ significantly at ($P < 0.05$)

Table 2. HPLC test results comparing non-thermal techniques for their efficiency in degrading Afla B1, Ochra A, and Fum B1 from poultry feeds after 60 min of exposure

	Afla B1		Ochra A		Fum B1	
Type of exposure	**C (µg/kg)**	**D (%)**	**C (µg/kg)**	**D (%)**	**C (µg/kg)**	**D (%)**
Control	21.84	0.00±0.00 ^{d}	149.0	0.00±0.00 ^{d}	6614	0.00±0.00 ^{d}
APCD	3.280	84.9±1.00 ^{a}	19.21	87.1±0.56 ^{a}	904.7	86.3±0.70 ^{a}
UVC w/ O_3	6.970	68.1±0.29 ^{b}	37.18	75.8±0.21 ^{b}	1641	75.2±0.50 ^{b}
PTW	13.82	36.7±1.15 ^{c}	86.81	41.7±1.15 ^{c}	3634	45.1±0.23 ^{c}

APCD: atmospheric pressure corona discharge, UVC w/ O_3: ultraviolet C with ozone, PTW: plasma-treated water, Control: without exposure. C: Concentration, D: Degradation represented as mean ± SEM (n=3). Vertical superscript letters that are not identical differ significantly at ($P < 0.05$)

The ELISA results were confirmed by HPLC analysis for the determination of target mycotoxins in samples subjected to non-thermal treatments for the longest period of time. According to HPLC analysis, the results regarding the estimation of mycotoxins in samples pre- and post-nonthermal treatments displayed lower mean concentrations with higher degradation levels than those observed in ELISA, where APCD had significantly higher levels ($P < 0.05$) of mycotoxins degradation, then UVC w/ O_3, whereas PTW displayed the lowest degradation levels. Regarding Afla B1, samples exposed to APCD, UVC w/ O_3, and PTW techniques for 60 min showed degradation levels of 84.99, 68.1, and 36.7%, for Ochra A 87.1, 75.8, and 41.7%, and for Fum B1 86.3, 75.2, and 45.1%, respectively (**Table 2**).

The application of NTTs to poultry feed samples for 10 min revealed no adverse impact on feed components and peroxide values. No significant differences ($P > 0.05$) were observed in the ash, protein, carbohydrate, and fat contents of the feeds as well as the values of the extracted fat peroxide after 10 min of treatment with APCD, UVC w/ O_3, and PTW (**Table 3**).

There were also no negative effects on feed composition with insignificant variations ($P > 0.05$) among NTTs with respect to their effects on feed composition after 20 min of exposure. A significant increase ($P < 0.05$) in peroxide value was indicated in feed samples subjected to UVC w/ O_3 treatment for 20 min compared to samples subjected to APCD and PTW, which offered an increment in peroxide values that did not vary significantly compared to untreated samples (**Table 3**).

The results of 60-min treatment of feed samples with NTTs displayed a significant reduction ($P < 0.05$) in ash followed APCD treatment. A significant reduction ($P < 0.05$) in protein content was found in samples subjected to APCD, UVC w/ O_3, and PTW compared to not subjected ones, where the decrease in protein was significantly higher ($P < 0.05$) followed UVC w/ O_3 and APCD treatments. Not-

significant variations in carbohydrate content were indicated between untreated and APCD, UVC w/ O_3, and PTW treated samples. Fat declined significantly ($P < 0.05$) in samples exposed to APCD and UVC w/ O_3. A significant increment ($P < 0.05$) in PVs of the fat was reported followed 60 min non-thermal treatments, with APCD recording the highest value, followed by UVC w/ O_3, and then PTW (**Table 3**).

Table 3. Comparison of non-thermal techniques in terms of their effect on feed components and peroxide values after 10, 20, and 60 min of treatment

Exposure time (min)	Type of exposure	Ash (%)	Protein (%)	Carbohydrate (%)	Fat (%)	PV (meq/kg)
10	Control	9.28±0.031	20.88±0.015	56.16±0.010	2.89±0.015	3.85±0.010
	APCD	9.30±0.150	20.84±0.015	56.14±0.015	2.92±0.015	3.84±0.025
	UVC w/ O_3	9.27±0.015	20.82±0.015	56.14±0.006	2.85±0.025	3.88±0.006
	PTW	9.30±0.017	20.83±0.021	56.16±0.006	2.85±0.010	3.89±0.021
20	Control	9.28±0.031	20.88±0.015	56.16±0.010	2.89±0.015	3.85±0.010 ^{b}
	APCD	9.31±0.021	20.82±0.006	56.15±0.010	2.91±0.015	3.87±0.012 ^{b}
	UVC w/ O_3	9.28±0.015	20.84±0.021	56.15±0.010	2.88±0.015	3.94±0.020 ^{a}
	PTW	9.27±0.012	20.82±0.021	56.15±0.012	2.84±0.015	3.89±0.015 ^{b}
60	Control	9.28±0.031 ^{a}	20.88±0.015 ^{a}	56.16±0.010	2.89±0.015 ^{a}	3.85±0.010 ^{d}
	APCD	9.15±0.010 ^{b}	20.66±0.015 ^{c}	56.17±0.015	2.74±0.021 ^{b}	4.21±0.010 ^{a}
	UVC w/ O_3	9.27±0.021 ^{a}	20.64±0.006 ^{c}	56.16±0.015	2.73±0.020 ^{b}	4.13±0.006 ^{b}
	PTW	9.24±0.006 ^{a}	20.83±0.012 ^{b}	56.16±0.010	2.84±0.010 ^{a}	3.91±0.017 ^{c}

APCD: atmospheric pressure corona discharge, UVC w/ O_3: ultraviolet C with ozone, PTW: plasma-treated water, Control: without treatment. Data are presented as mean ± SEM (n=3).. Vertical superscript letters that are not identical differ significantly at ($P < 0.05$)

DISCUSSION

Non-thermal techniques are among the emanating strategies recently spotlighted in the food sciences for mycotoxins destruction, due to their unique provisional traits (**46,47**).

Comparison of NTTs (APCD, UVC w/ O_3, and PTW) in their efficiency in degrading Afla B1, Ochra A, and Fum B1 from feed offered significant variations among them in the levels of mycotoxins degradation with corona discharge under atmospheric pressure showing the highest levels of mycotoxins degradation, followed by ultraviolet C with ozone, whereas plasma treated water showed the lowest levels of degradation. The comparative effectiveness of these three NTTs in degrading mycotoxins has not been documented by other research studies. However, the supremacy of cold atmospheric pressure plasma (CAP) over UVC radiation in the degradation of mycotoxins has only been notarized in one research study (**48**). CAP recorded degradation levels of Afla B1 and Fum B1 of over 99.99% and 90%, respectively, while UVC recorded values of approximately 50% and 63%, respectively, after 8 min of exposure on clean glass coverslips. APCD outperforms UVC w/ O_3 primarily through generation a multitude of RONS as mentioned earlier (**21, 22**). The limited quantity of RONS generated in water after plasma treatment may explain the lower efficiency of PTW compared with APCD in degrading mycotoxins, a fact that has also been confirmed in another research (**49**).

Confirmation of the ELISA findings using HPLC assay for the quantification of mycotoxins in samples exposed to non-thermal treatments showed similar results, with APCD outperforming other non-thermal techniques in its degradation levels, followed by UVC w/ O_3, whilst PTW showed the lowest degradation levels. HPLC analysis demonstrated that mean concentrations of target mycotoxins were less than those calculated in ELISA with destruction levels more than those estimated in ELISA. Identical findings have been proven previously (**36**). Nesic et al. (**50**) exhibited that the mean aflatoxin concentration (7.5 µg/kg) using ELISA was higher than the mean measured using HPLC (6.2 µg/kg), whilst for ochratoxin a lower mean concentration (5.8 µg/kg) was recorded using ELISA than the mean measured using HPLC (6.5 µg/kg).

Increasing the exposure time of feed samples to non-thermal treatments negatively affects feed composition and peroxide values. At 10 and 20 min of subjecting feed to APCD, UVC w/ O_3, and PTW, feed composition was not affected significantly ($P < 0.05$), while peroxide values increased significantly ($P < 0.05$) from 3.85 meq/kg to 3.94 meq/kg after UVC w/ O_3 application for 20 min. After 60 min of non-thermal treatments, the decrease in protein content after APCD and UVC w/ O_3 treatments was higher significantly ($P < 0.05$) compared to PTW. Also, a significant decline ($P < 0.05$) in fat followed APCD and UVC w/ O_3 treatments and in ash followed APCD treatment was also noted. A significant increase ($P < 0.05$) in peroxide values was observed followed non-thermal treatments, with APCD showing the highest increase in PVs, followed by UVC w/ O_3, while PTW showed the lowest increase in PVs. Feed components were not adversely affected after treatment with these NTTs for short periods. Increasing exposure time and/ or applied voltage in the case of CP treatment and/ or UVC intensity in the case of UVC w/ O_3 treatment may negatively affect the nutritional components (**49,51-53**). Increasing the exposure time during CP application result in an increment in the generated reactive species that interact with food cells (**49,54**). As mentioned earlier, the restricted amount of RONS in PTW may interpret its lower negative impacts on feed quality compared to APCD that produced a large number of RONS (**49**).

Comparative studies explaining the effects of APCD, UVC w/ O_3, and PTW on poultry feed composition and PVs of feed fats have not been indicated in other research studies, although the effect of each method alone on some food and feed components and PVs has been documented in other

researches. Furthermore, no studies have been conducted to investigate the synergistic effect of UVC w/ O_3 on food and feed components.

Concerning ash content, non-thermal exposure for 10 and 20 min did not affect feed ash content, while a 60-min exposure presented a significant decrease ($P < 0.05$) after APCD treatment. Other researches have indicated a not significant alteration ($P < 0.05$) in ash followed handling wheat with CP for 30 seconds (**55**) and maize treatment with ozone at a level of 13.5 mg/l for 60 h (56). Celik et al. (**57**) demonstrated a decline in the broiler feed ash content after treating with ozone at diverse levels and times. Sarangapani et al. (**58**) referred to significant differences in the black gram ash content in low pressure plasma treated samples compared to untreated ones.

Regarding protein content, after 10 to 20 min of exposure to NTTs, protein was not negatively affected. After 60 min of exposure, a significant decline in protein was noted, although it was still within the standard protein levels recommended for poultry feed (20% for broiler grower), in order to stimulate growth and egg production on a large-scale, uphold the immune system, and assure healthy feather upgrowth (**59**). No significant changes in protein content were reported after treating flour with 45 mg/l O_3 for 60 min (**60**), treating maize with 13.5 mg/l O_3 for up to 60 h (**56**), and treating bread with CP for 2 and 10 min in the majority of cases (**61**). Garg et al. (**62**) observed a significant decline in protein after 12 h of UVC treatment of peanuts from 26.81% to 25.25%. Ali and Abdallah (**63**) also showed that treating nuts with ozone gas for 180 min at a concentration of 4 ppm resulted in a 38.4% reduction in the protein content. Whereas Sarangapani et al. (**58**) referred to a significant increase in black gram content of protein from 23.15% to amounts ranging from 23.21 to 23.99% after plasma processing which was in contrast to our results. In CP, the substantial role of atomic oxygen and hydroxyl radicals on proteins may modify the protein molecular properties leading to differences in protein content (**64**). In addition, ROS in PTW can lead to changes in protein conformation due to specific bond dissociation and side chain modification (**65**).

Poultry feed carbohydrate content was insignificantly influenced after 10, 20, and 60 min of exposure. The findings were in line with those observed by (**62**) which indicated that peanuts carbohydrate content was not adversely affected after UVC irradiation for 12 h at a distance of 15 cm to record a value of 14.98% compared to the untreated sample (15.92%). Wang et al. (**66**) demonstrated that treating wheat grains with ozone (75 mg/l) for 90 min also recorded non-significant differences in starch content. In addition, the carbohydrate was not affected after PTW application as shown in other research studies (**67-69**). The findings were incompatible with the findings of (**58**) which indicated that there were significant variations in the black gram carbohydrate content after plasma processing. Furthermore, a decline in the carbohydrate content of nuts (42.7%) after processing with 4 ppm O_3 gas for 180 min was also indicated (**63**).

The fat content of poultry feed was not adversely affected after 10 and 20 min of exposure to NTTs, and was significantly decreased after exposure to APCD and UVC w/ O_3 for 60 min. The prominent role of fats is not limited to simply providing energy but also embraced numerous important physiological and practical aspects. However, despite the significant decrease in fats after 60-min exposure to APCD and UVC w/ O_3, they remained within the standard levels established for poultry feed (which are usually between 2% and 7% or more for broiler grower) (**59**). These results were in line with those observed by Mohammadi et al. (**55**) who reported non-significant variations in the wheat fat content after CP exposure for 30 sec. A reduction in the fat content of peanut after exposure to UVC at 15 cm distance for 12 h (**62**), nuts fat content after exposure to ozone gas (4 ppm) for 180 min (**63**) and bread fat content (about 28%) after exposure to CP for 2 min (**61**) were reported. The results showed a significant increment in PVs of poultry feed fat after UVC w/ O_3 treatment for 20 min and after APCD, UVC w/ O_3, and PTW treatments for 60 min, although the values were still below 5 meq/kg. Wealleans et al. (**70**) classified the oxidative quality of fats and oils into 4 categories, among which when PV < 5 meq/kg indicates no oxidation. According to this classification, the three non-thermal treatments for up to 60 min that were adopted in this study preserved the quality and freshness of poultry feed fats. The increment in PVs has been documented in other studies, as in the research performed by Jung et al. (**71**) which demonstrated that peroxide values increased after storage of PTW-processed sausage. Perna et al. (**52**) also showed a significant increase in PV of cream from 0.29 meq/kg in unexposed samples to 4.09 meq/kg after one hour of ozonation. The synergistic effect of UVC w/ O_3 on PV was clarified only by Li et al. (**30**) in peanut oil which was not significantly affected after 30 min of processing. The reduction in fat content with increasing PVs may be due to lipid peroxidation by ROS generated in CP and PTW (**54,72**), as well as the synergistic effect of UVC with O_3 (**52,73**).

The apparent variability in the efficiency of the NTTs under study in detoxifying mycotoxins and their impact on food quality between the present study and others is based on several criteria such as the type of the technique and handling conditions, in addition to the type of mycotoxin and food matrix (**30,49,74**).

In the present study, the analysis was limited to monitoring the degradation/removal efficiency of the target mycotoxins, and the formation and toxicity of possible secondary metabolites were not experimentally investigated. Therefore, this aspect represents a limitation of our work.

Ultimately, the study spotlighted the efficiency of NTTs, in particular APCD and UVC w/ O_3, in degrading Afla B1, Ochra A, and Fum B1 in feeds while preserving their nutritional components and oxidative properties. These recent findings have paved the way for future studies to perform a comprehensive identification and toxicological assessment of mycotoxin degradation byproducts to fully

evaluate the safety of the detoxification process, taking into account the specificity of each mycotoxin in each application and product, and to adopt these NTTs for large-scale applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: HSA. Methodology: HSA; TND; QA. Investigation: HSA. Supervision: QA (APCD, UVC w/ O_3, and PTW experiments); TND. Formal analysis: HSA; TND; QA. Writing – Original Draft: HSA. Writing – Review & Editing: TND; QA. All authors have read and approved the final version of the manuscript.

ARTIFICIAL INTELLIGENT DECLARATION

The authors declare that they are responsible for the accuracy and integrity of all content of the manuscript, including part generated by AI, and it is not used as a co-author.

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مقارنة كفاءة بعض التقنيات غير الحرارية في إزالة سمية سم الأفلا B1 وسم الأوكرا A وسم الفومونيسين B1 من أعلاف الدواجن

هبة صلاح الدين النعيمي[١]، تمارة ناطق داؤود[٢]، قيس ذنون الجواري[٣]

[١]فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق، [٢]فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق، [٣]قسم الالكترونيك، كلية هندسة الالكترونيات، جامعة نينوى، الموصل، العراق

الخلاصة

لقد تم تسليط الضوء مؤخرًا على تفريغ الهالة المتولدة عند الضغط الجوي، والأشعة فوق البنفسجية نوع C مع الأوزون، والماء المعالج بالبلازما في صناعة الأغذية، كتقنيات غير حرارية لإزالة السموم الفطرية، نظرًا لخصائصها الفريدة. تم التحري عن كفاءة هذه التقنيات في إزالة سمية كل من سم الأفلا B1، وسم الأوكرا A، وسم الفومونيسين B1 من أعلاف الدواجن وتأثيراتها على جودة الأعلاف. خضعت عينات الأعلاف للمعاملة بتفريغ الهالة تحت الضغط الجوي، والأشعة فوق البنفسجية نوع C مع الأوزون، والماء المعالج بالبلازما لمدة ١٠ و٢٠ و٦٠ دقيقة. خُددت تراكيز السموم الفطرية باستخدام اختبار الممتز المناعي المرتبط بالإنزيم التنافسي (ELISA)، وتم التحقق من النتائج باستخدام تقنية الاستشراب السائل عالي الأداء (HPLC). تم اعتماد الطرق التحليلية القياسية لتحليل مكونات العلف وتحديد قيم البيروكسيد. أدى تعريض العينات لتفريغ الهالة تحت الضغط الجوي، والأشعة فوق البنفسجية نوع C مع الأوزون، والماء المعالج بالبلازما لمدة ١٠ دقائق إلى تحلل سم الأفلا B1 إلى مستويات بلغت ٤٦,٦ و٣٨,٩ و٢٨,٩٪، وسم الاوكرا A إلى ٤٩,٨ و٣٥,٩ و٢٩,٩٪، وسم الفومونيسين B1 إلى ٥٨,٦ و٤٢,٦ و٣٥,٩٪ على التوالي، ولمدة ٢٠ دقيقة إلى تحلل سم الأفلا B1 الى مستويات بلغت ٥٧,٧ و٤٦,٦ و٣٢,٩٪، وسم الاوكرا A إلى ٦٨,٩ و٤٥,٣ و٣٨,٥٪، وسم الفومونيسين B1 إلى ٧٥,٧ و٤٩,٩ و٤١,٦٪ على التوالي، ولمدة ٦٠ دقيقة الى تحلل سم الأفلا B1 إلى مستويات بلغت ٨٣,٢ و٦٥,٧ و٣٣,٨٪، وسم الاوكرا A إلى ٨٤,٢ و٧٣,٦ و٤٠,٧٪، وسم الفومونيسين B1 إلى ٨٤,٨ و٧١,٢ و٤٣,٤٪ على التوالي. تضمن الاستنتاج الرئيسي للدراسة بإمكانية اعتماد كل من تقنية تفريغ الهالة والأشعة فوق البنفسجية نوع C مع الأوزون بنجاح لتحلل كل من سم الأفلا B1، وسم الأوكرا A، وسم الفومونيسين B1 بكفاءة في الأعلاف مع الحفاظ على جودتها. وفقًا لتأثير التقنيات غير الحرارية على جودة الأعلاف، يمكن ترتيبها على النحو التالي: تفريغ الهالة تحت الضغط الجوي> الأشعة فوق البنفسجية نوع C مع الأوزون > الماء المعالج بالبلازما.

الكلمات المفاحية: سم الأفلا B1، سم الفومونيسين B1، سم الأوكرا A، علف الدواجن، تقنية غير حرارية