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Ultraviolet C and Ozone Application for Detoxification of Aflatoxin B1, Ochratoxin A, and Fumonisin B1 in Poultry Feeds



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Abstract

THE EFFECTIVENESS of the synergistic effect of ultraviolet C irradiation with ozone L (UVC+O₂) for aflatoxin B1 (AFB1), ochratoxin A (OTA), and fumonisin B1 (FB1) detoxification in naturally contaminated poultry feeds with their impacts on the feed nutritional components were investigated. Feed samples were exposed to UVC+O, for six durations (10, 20, 30, 60, 120, and 180 min) at three distances (15, 30, and 60 cm) with UVC doses ranging between 577.8 to 10400.4 mJ/cm² at 15 cm, 397.8 to 7160.4 mJ/cm² at 30 cm, and 50.4 to 907.2 mJ/cm² at 60 cm at a constant O₃ concentration of 10 ppm. Mycotoxin levels were determined by competitive Enzyme-Linked Immunosorbent Assay (ELISA) and results were confirmed by High-Performance Liquid Chromatography (HPLC). Feed component analysis with peroxide values (PVs) appreciation was executed according to standard analytical methods. A significant increase (P<0.05) in degradation percentages of AFB1, OTA, and FB1 was recorded with increasing exposure time and decreasing distances to reach values of 80.94, 84.07, and 83.6% at 15 cm, 78.49, 83.89, and 83.89% at 30 cm and 67.9, 74.76, and 72.89% at 60 cm, respectively after 180 min of treatment. OTA and FB1 showed degradation levels significantly higher (P<0.05) than those recorded for AFB1. Feed components were intermediately affected by UVC+O3 treatment. Feed fats were still having good quality depending on PVs estimation. In brief, the efficiency of the synergistic effect of UVC+O₃ in AFB1, OTA, and FB1 detoxification from poultry feed with a moderate impact on feed quality.

Keywords: Feed quality, Mycotoxins, Ozone, Ultraviolet C.

Introduction

Mycotoxigenic molds that exist throughout the environment may infect human foods and animal feeds, especially crops, resulting in mycotoxins entering the food chain [1-3]. Mycotoxins encompass a wide range of secondary toxic metabolites produced by molds in various food and feed ingredients during pre- and post-harvest periods. Aflatoxin B1 (AFB1), ochratoxin A (OTA), and fumonisin B1 (FB1) are categorized as the most commonly encountered, hazardous, and

economically important mycotoxins [4, 5]. Human exposure to these mycotoxins can happen either directly through consuming contaminated food or indirectly through consuming foods of animal origin gained from animals fed on contaminated feeds, resulting in many pathological influences such as hepatotoxic, nephrotoxic, immunotoxic, neurotoxic, carcinogenic, mutagenic and teratogenic effects [5-7].

Physical, chemical, and biological technologies have been developed to detoxify

mycotoxins in foods and feeds [8, 9]. Recently, a lot of attention has been given to non-thermal physical technologies due to their convenience with a wide range of foods and feeds, inexpensive, less timewasting with mild impact on food quality [10].

Ultraviolet (UV) irradiation is an effective non-thermal physical technology used to degrade chemical contaminants through photolysis [11]. UV rays have a wavelength extending from 100 to 400 nm. The four main forms of UV rays include UVA (315–400 nm), UVB (280–315 nm), UVC (200–280 nm), and vacuum ultraviolet (VUV) (100–200 nm). It is worth mentioning that the bond energies of most mycotoxins coincide with the wavelengths within the UV spectrum [12]. Several researches clearly demonstrated the effectiveness of UV irradiation in mold and mycotoxins decontamination, especially in UVC form [13, 14].

Ozone (O₃), a triatomic allotrope of oxygen, is a highly reactive gas with a high oxidation/reduction potential which is higher than other prevalent food industry oxidants. Ozone gas generation occurs when the atmospheric air is subjected to a high-energy source such as UV, corona discharge, or electrolysis [15]. A significant mycotoxin degradation has been reported in both naturally and artificially contaminated samples with mycotoxins after ozonation [16, 17]. The mechanism by which mycotoxin detoxification by ozonation happens is attributed to ozone reaction with the functional groups in the mycotoxin molecules [18].

Low-pressure UV lamps with shortwaves can emit UV light at two peaks in the UV light band, one at 254 nm (UVC) which is indicated as a "germicidal", and the other at 185 nm (VUV) which is indicated as an "ozone producing". VUV light interacts with oxygen to splinter it into atomic oxygen which is a highly unstable atom that coalesces with oxygen to form ozone, a powerful oxidant [19]. The synergistic effect of both UVC and O₃ for mycotoxin detoxification was investigated only by Li et al. [20] through employing a dielectric barrier discharge ozone generator and a UVC lamp.

The efficiency of low-pressure UVC lamps that produce ozone in detoxifying mycotoxins has not been studied yet. Thus, the research intended to evaluate the effectiveness of ozone-producing UVC lamps in AFB1, OTA, and FB1

detoxification from poultry feeds, determine the parameters that are effective in detoxification, as well as investigate the impact of the treatment on the nutritional components and PVs of the feed.

Materials and Methods

Feed samples

Pelleted poultry feed samples were gained from poultry feed manufactories and poultry farms in Nineveh Province, northern Iraq from April to September 2022. The samples were homogenized and quartered to gain 1 kg of representative samples. The samples were milled utilizing a laboratory mill and sieved through NO. 18 mesh sieve. The samples that exhibited AFB1, OTA, and FB1 co-occurrence at the highest levels were subjected to UVC+O₃ application.

UVC+O, application

Application of UVC+O, was performed in a closed chamber with a dimension of (65×40×115 cm) fitted with an ozone-generating UVC lamp of 38 W (360° UVC sterilization lamp, China) in a temperature-controlled room at 25°C with no external lighting. The lamp is equipped with two high-quality UVC tubes with ozone production from short UV light wavelengths, producing UV light with two peaks at 254 nm and 185 nm. The lamp was provided with a remote control with a ten-second delay for safety. The feed sample was disseminated uniformly on a tray, a 20 cm diameter, in a thin layer (about 0.1 cm). The tray was placed on the chamber shelves which were adjusted at three vertical distances of 15, 30, and 60 cm below the UVC lamp. The exposure times were set for six durations (10, 20, 30, 60, 120, and 180 min). A digital UVC meter was used for measuring UVC radiation intensity (RGM-UVC meter, China). The UVC lamp was allowed to warm up for 10 min before use. The UVC intensity measured at a 15 cm distance away from the lamp amounted to 0.963 mW/cm², a 30 cm distance of 0.663 mW/cm², and a 60 cm distance of 0.084 mW/cm². The UVC dose was estimated from the equation D = I * t.

Where:

D: UVC dose (mJ/cm²), I: UVC radiation intensity (mW/cm²), and t: time (sec).

The doses of the UVC at the six-time durations investigated ranged between 577.8 to 10400.4 mJ/cm² at 15 cm, 397.8 to 7160.4 mJ/cm² at 30 cm, and 50.4 to 907.2 mJ/cm² at 60 cm (Table 1).

Ozone concentration was kept at the level of 10 ppm through a suction fan controlled by an ozone sensor which was adjusted at the particular concentration. A circulation fan was used for efficient $\rm O_3$ circulation. To verify the ozone concentration inside the chamber, a portable $\rm O_3$ gas detector of 100 ppm (China) was utilized every 10 min. The sample temperature was calculated using a digital thermometer. All experiments were conducted in triplicate.

AFB1, OTA, and FB1 quantification in poultry feeds by ELISA

Concentrations of AFB1, OTA, and FB1 in poultry feed samples before and after UVC+O₃ treatment were measured using ELISA kits (Elabscience Biotechnology Inc., USA). The kit is based on the competitive ELISA for detecting of the aforementioned mycotoxins in feed samples.

Feed sample pretreatment was accomplished according to the kit manufacturer's instructions.

Competitive ELISA procedures for AFB1, OTA, and FB1 detection were carried out according to the instructions of the kit manufacturer. The analysis was conducted on both the standards and the samples in duplicate. The optical density (OD) values were specified using a microplate reader (HumaReader HS, Germany) at a wavelength of 450 nm.

HPLC analysis for ELISA results confirmation

Chromatographic analysis was performed on feed samples that exhibited co-occurrence of AFB1, OTA and FB1 at the highest levels for UVC+O₃ application. Additionally, samples that showed the highest mycotoxin degradation levels after treatment were analyzed to confirm the ELISA results.

AFB1 estimation by HPLC was conducted according to Kim et al. [21]. AFB1 standard was gained from Sigma-Aldrich (St-Louis, MO, USA). Acetonitrile-methanol (1:1 v/v) of HPLCgrade was used for preparing stock solution of AFB1. The initial step involved the extraction of AFB1 from ground poultry feed samples. Samples clean-up was performed utilizing Sep-Pak® silica cartridges (Waters Corporation, Milford, MA, USA). A mixture of chloroform and acetone in a 9:1 ratio (v/v) was utilized for the elution of AFB1. After derivatization, the lower phase of the derivatized solution was obtained for HPLC (Shimadzu Corp., Japan). The column used was a Nucleodur ® C18 column (4.6 × 250 mm, Germany). The wavelength of the fluorescence

detector was set to 360 nm for excitation and 440 nm for emission (Shimadzu Corp., Japan). The mobile phase used was a mixture of methanol, acetonitrile, and water in a ratio of 17:17:70 (v/v/v) with a flow rate of 1 ml/min.

Confirmation of OTA existence in poultry feed samples by HPLC was conducted as recommended by Nesheim et al. [22]. OTA standard was obtained from Sigma-Aldrich (St-Louis, MO, USA). The stock solution of OTA was prepared in benzene-acetic acid (99:1 v/v). After extraction of mycotoxin, the bicarbonate extract was cleaned up using a Sep-Pak® C18 cartridge (Waters). To elute OTA, we used ethyl acetate-methanol-acetic acid (in the ratio of 95:5:0.5). HPLC with fluorescence detection set at 333 nm for excitation and 460 nm for emission was used for OTA estimation. The mobile phase consisted of acetonitrile-water-acetic acid (in the ratio of 99:99:2 v/v/v) and the flow rate was adjusted to 1 ml/min.

FB1 standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution was prepared in a 1:1 v/v mixture of acetonitrile and water. FB1 was extracted from poultry feed samples for HPLC analysis using the method described by Shephard et al. [23]. After extracting FB1, an aliquot was cleaned up using strong anion extraction (SAX) cartridges (InertSep SAX, GL sciences, Japan). FB1 was eluted using acetic acid in methanol. The HPLC injection was done within one to two minutes after derivatization. The fluorescence detector was set to excitation and emission wavelengths of 335 and 440 nm, respectively. The mobile phase consisted of methanol: 0.1 M sodium dihydrogen phosphate (80:20), and the flow rate was set to 1 ml/min.

Mycotoxin degradation percentages and mycotoxin degradation kinetics

After applying UVC+O₃, the degradation percentages of mycotoxins in poultry feeds were measured using the following equation:

Mycotoxin degradation (%) = $(1-C_t/C_0) \times 100$ Where:

 C_t = the concentration of mycotoxin at the time (t).

 C_0 = the initial concentration of mycotoxin at zero time.

The degradation kinetics of mycotoxins were modeled using the exponential decay model [24], which can be fit as follows:

$$y = y_0 + A_1 * exp(-(x-x_0)/t_1)$$

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Where:

 $y \equiv C_t$; $y_0 \equiv C_0$; $x \equiv t$; A_1 , x_0 and t_1 are constants.

Evaluate the impact of $UVC+O_3$ on the nutritional components and PVs of poultry feed

The combined impact of UVC and O, on the nutritional components and PVs of poultry feeds was inspected. The moisture content of the feed was determined using the AOAC official method 930.15 by estimating water loss-on-drying at 135 °C for 2 hours [25]. Total carbohydrates were measured using the phenol-sulphuric acid method described by DuBois et al. [26]. Crude fat was estimated using a Soxhlet system following the AOAC method 920.39 [27]. The Kjeldahl method, as stated by AOAC method 984.13 [28], was used for crude protein determination. Ash was measured according to AOAC Official method 942.05 [29]. Finally, peroxide values in the feed fats were estimated using method 965.33 recommended by AOAC [30].

Statistical analysis

The data was analyzed using the Two Way Analysis of Variance procedure of the Sigma Stat for Windows Version 3.10. To compare the means at a significance level of *P*<0.05, Duncan's Multiple Range Test was conducted as described by Steel and Torri [31].

Results

AFB1, OTA and FB1 degradation after $UVC+O_3$ application

Concerning the degradation influences of UVC+O₃ on AFB1, OTA, and FB1 in samples of poultry feeds, results offered a decrease in the mean levels of targeted mycotoxins from 23.71, 164.66 and 6850.25 µg/kg in naturally co-contaminated samples to values of 4.52, 26.13 and 1123.44 µg/kg at 15 cm distance, 5.1, 26.39 and 1103.58 µg/kg at 30 cm distance and 7.61, 41.46 and 1857.1 µg/kg at 60 cm distance, respectively after 180 min of exposure to UVC intensity of 0.963 mW/cm², 0.663 mW/cm², and 0.084 mW/cm² at 15, 30, and 60 cm, respectively with a constant O₃ concentration of 10 ppm. A significant increase (P<0.05) in AFB1, OTA, and FB1 degradation levels in poultry feed samples was recorded after 180 min from UVC+O₃ exposure, as they outreached values of 80.94, 84.07 and 83.60% at 15 cm distance, 78.49, 83.89 and 83.89% at 30 cm distance and 67.90, 74.76 and 72.89% at 60 cm distance, respectively. Moreover, results illustrated that OTA and FB1 exhibited the highest degradation levels at the Egypt. J. Vet. Sci. Vol. 56, No. 4 (2025)

three examined distances, where no significant differences (P<0.05) were reported between them. While AFB1 recorded the lowest degradation levels which were significantly different (P<0.05) from those reported for OTA and FB1 (Tables 2-4).

The degradation levels of examined mycotoxins were significantly increased (P<0.05) with increasing exposure time and decreasing distances after UVC+O₃ treatment, except OTA which showed insignificant differences (P<0.05) in its degradation at 15 and 30 cm distances (Tables 5-7).

After 180 min of UVC+O₃ treatment, the feed sample temperature slightly increased from 25 °C to 28.4, 27.5, and 26.5 °C at the first, second, and third distances, respectively.

The degradation rate of mycotoxins after UVC+O₃ treatment following an exponential model was well fitted (Adj. R²>0.91) for AFB1, OTA, and FB1 at the three studied distances (Figures 1-3).

HPLC analysis was conducted for ELISA results confirmation for samples submitted to UVC+O3 treatment and samples showed the highest degradation levels. The results of HPLC analysis exhibited AFB1, OTA, and FB1 presence in poultry feed samples before and after UVC+O, treatment at mean concentrations less than those recorded in ELISA with degradation levels higher than those measured in ELISA. According to HPLC results, AFB1, OTA, and FB1 co-occurred at mean concentrations of 21.84, 149 and 6613.5 respectively. These concentrations diminished after 120 and 180 min UVC+O, treatment to values amounted to 5.64, 25.22 and 1359.54 µg/kg and 3.67, 18.89, and 979.25 μg/kg, respectively at 15 cm and 5.37, 29.83, and 1618.41 µg/kg and 3.92, 22.07 and 950.11 μg/kg, respectively at 30 cm. The degradation percentages of AFB1, OTA, and FB1 were 74.18, 83.07, and 79.44% and 83.20, 87.32, and 85.19%, respectively after 120 and 180 min exposure at 15 cm and 75.41, 79.98 and 75.53% and 82.05, 85.19 and 85.63%, respectively after 120 and 180 min exposure at 30 cm (Tables 8 and 9).

 $UVC+O_3$ impact on feed nutritional composition and PVs

Results related to the analysis of feed components referred to a significant decrease (P<0.05) in moisture content of feed samples treated with UVC+O₃ with increasing exposure

time and decreasing distances. The moisture content decreased from 9.85% in untreated samples to reach values of 7.52, 7.73, and 8.07% after 180 min treatment at 15, 30, and 60 cm distances, respectively (Figures 4-6, Table 10).

According to the results presented, the protein content of poultry feeds showed a significant decrease (P<0.05) from 20.88% in untreated samples to values of 20.63, 20.69 and 20.81% in UVC+O₃ treated samples for 180 min at distances of 15, 30, and 60 cm, respectively. Additionally, the decrease in protein content increased significantly (P<0.05) with decreasing distances, as it amounted to 20.75, 20.80, and 20.85% at 15, 30, and 60 cm, respectively (Figures 4-6, Table 11).

Results related to the effect of UVC+O₃ treatment on the carbohydrate content of poultry feeds exhibited no significant differences (*P*<0.05) in content between untreated and treated samples at different time durations and distances (Figures 4-6, Table 12).

Regarding the ash content of poultry feeds, results showed insignificant differences (P<0.05) in their ash content during different time durations of exposure and distances. However, a significant decrease (P<0.05) in ash content was observed only at a 15 cm distance after 180 min of treatment to record a value of 9.19% in comparison with the untreated one (9.28%) (Figures 4-6, Table 13).

Fats of poultry feeds presented insignificant differences (P<0.05) in their contents after 20, 30, and 180 min of UVC+O₃ application at 15, 30, and 60 cm distances, respectively. After that, fat content decreased significantly (P<0.05) at the first and the second distances from 2.89% in untreated samples to reach values of 2.75 and 2.79%, respectively after 180 min of treatment. Also, results showed that fat contents decreased significantly (P<0.05) with decreasing distances, as they amounted to 2.81, 2.84 and 2.87% at 15, 30, and 60 cm, respectively (Figures 4-6, Table 14).

Results exhibited insignificant differences (P<0.05) in PVs after 10, 30, and 60 min of treatment at 15, 30, and 60 cm, respectively. Thereafter, PVs increased significantly (P<0.05) from 3.85 meq/kg in untreated samples to values of 4.25, 4.13 and 3.95 meq/kg after 180 min treatment at 15, 30, and 60 cm, respectively. Additionally, results revealed that PVs increased significantly (P<0.05) with decreasing distances,

where they recorded 4.04, 3.94 and 3.88 meq/kg at 15, 30, and 60 cm, respectively (Table 15).

Discussion

Despite the numerous attempts to prevent mold growth and mycotoxins contamination, mycotoxin residues in the food chain may persist as one of the great issues that are difficult to predict and prevent. Due to their significant risks to public health and their stability against many physical and chemical traditional strategies, several efforts have been made worldwide to develop novel, nonthermal, and environmentally friendly strategies for mycotoxin decontamination. UVC irradiation with ozone may exemplify an efficient strategy for mycotoxins detoxification by combining two mechanisms, UVC photooxidation, and the high oxidizing power of ozone [10, 15].

AFB1, OTA, and FB1 detoxification was documented by other researchers after the application of UVC [13, 32] or ozone [16, 17] alone. The combined effect of both UVC and O, for AFB1 detoxification in artificially contaminated peanuts (500 µg/l) was documented by Li et al. [20] who exhibited an AFB1 degradation rate of 79.01% after treatment with 5 mg/l ozone under UVC irradiation ($\lambda = 254$ nm, 350 μ W/cm²) for 30 min at 0.4 cm. Our results were in accordance to a certain extent these results with AFB1 degradation levels lower than these results after 30 min treatment. These differences in degradation levels may be attributed to the differences in the processing parameters and the differences in the pattern (natural or artificial) and level of matrix contamination with the mycotoxin, where a high initial AFB1 level leads to a high level of degradation [33]. The legal limits of AFB1 and OTA established by the European Commission (EC) in poultry feeds are 20 and 100 µg/kg, respectively [34, 35]. In accordance with these limits and to mycotoxin levels measured in our study, results stated that UVC+O, treatment for 10 min was enough to decrease AFB1 levels recorded at zero time to levels less than the established limit at the three examined distances. Whereas, 20 min UVC+O₃ treatment was sufficient to reach OTA legal limit at 15 and 30 cm and 30 min at 60 cm. No maximum level was set by the EC related to the legal limit of FB1 alone in poultry feeds, but the maximum limit for FB1+ FB2 together in poultry feeds which had been set by EC was 20000 μg/kg [34]. Poultry feed samples subjected to UVC+O3 treatment showed FB1 levels lower than the maximum permissible limit. Our results clarified that OTA and FB1 showed the highest degradation levels, whereas AFB1 exhibited the lowest degradation levels. These results were in agreement with the results mentioned by Sousa [32] which indicated that the degradation of OTA was higher than AFB1 degradation after UVC treatment of both aqueous and corn slurry samples artificially contaminated with these mycotoxins. Hojnik et al. [36] demonstrated that FB1 degradation level by UVC was higher than that recorded for AFB1, which was consistent with our results.

The degradation levels of studied mycotoxins were significantly increased (P < 0.05) with increasing exposure time and decreasing distances after UVC+O3 application. Decreasing the distance between the UVC lamp and the treated sample also resulted in increasing degradation level of aflatoxin in peanuts with increasing exposure time as showed by Garg et al. [37] who reported aflatoxin reduction levels of 99.1 % (350 ppb to 3 ppb) and 97.4% (350 ppb to 8 ppb) after treatment with UVC irradiation for 12 h at 15 cm (648 KJ/m²) and 30 cm (432 KJ/m²) distances, respectively. Li et al. [20] elucidated that AFB1 degradation levels in peanuts were increased in a time-dependent manner, particularly during the first 30 min of UVC+O₃ application (79.01%), which could be attributed to ozone saturation and the inability of UVC irradiation to penetrate peanuts and promote a further decrease in AFB1 level. In our study, similar results to a certain degree were presented where approximately 40-60% of mycotoxins degradation happened during the first 30 min of treatment while approximately 25-30% happened during the last two and a half hours of UVC+O3 treatment. Treating crushed feed samples, as demonstrated in our study, increased the surface area which in turn increased mycotoxins exposure to UVC irradiation resulting in a greater degradation with increasing exposure time in comparison to treating uncrushed samples.

Results clarified a slight increase in feed samples temperature after UVC+O₃ treatment for 180 min. Poultry feed temperature was also not significantly changed after UVC treatment (0.1 mW/cm²) at a distance of 25 cm over the feed sample [13].

HPLC analysis showed the presence of AFB1, OTA, and FB1 in poultry feed samples at mean levels less than those estimated in ELISA with degradation levels higher than those recorded in ELISA. The results of the HPLC analysis

were consistent with the results recorded by Alshawabkeh et al. [38].

Regarding UVC+O, impact on feed nutritional composition, the significant decrease (P<0.05) in feed moisture content after UVC+O₃ application demonstrated in our study was in line with the results mentioned by Li et al. [20] which showed a significant decrease (P<0.05) in peanuts moisture content from 7.49% to 7.22% after 60 min of UVC application only. Peanuts treatment with UVC in combination with 3, 5, and 7 mg/l ozone for 60 min led to a greater decrease in moisture content to values of 6.93%, 6.83%, and 6.68%, respectively, which were significantly lower (P < 0.05) than that recorded after UVC treatment only. While Sobeli et al. [39] reported an insignificant effect (P > 0.05) of pulsed UVC radiation (0.525, 1.05, 2.1 and 4.2 J/cm²) on total moisture content of beef loin steaks. Furthermore, Ribeiro et al. [17] exhibited insignificant change (P<0.05) in the moisture content of maize after treatment with ozone at a level of 13.5 mg/l for up to 60 h. In our study, the slight increase in sample temperature may contribute slightly to the decrease in the moisture content due to sample water evaporation. The major role in low moisture content may be attributed to O₃ decomposition that results in heat release and an increase in the temperature of air leading to sample drying acceleration. In addition, the vapor created from the binding of O, with free water of nutritional materials can participate in declining moisture content [20].

There are no studies that investigated the synergistic effect of UVC+O₂ on protein content in foods and feeds. However, the effects of the application of UVC or O3 alone on protein content were inspected in many researches. Results were in agreement with the results found by Garg et al. [37] which pointed out that the decline in protein content of peanuts after UVC irradiation increased with decreasing distances, as it amounted to 26.81% in untreated samples to reach values of 25.25% and 26.12% after 12 h of treatment at 15 and 30 cm, respectively. On the other hand, the results were incompatible with the results presented by Sobeli et al. [39] which exhibited an insignificant effect (P > 0.05) of pulsed UVC radiation (0.525, 1.05, 2.1 and 4.2 J/ cm²) on the total protein content of beef loin steaks. Likewise, Ribeiro et al. [17] demonstrated that maize treatment with 13.5 mg/l ozone for up to 60 h led to an insignificant change (P<0.05) in the protein content.

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Also, no studies referred to the combined effect of UVC+O₃ treatment on the carbohydrate content. Results were in accommodate with the results obtained by Garg et al. [37] which demonstrated an insignificant effect (*P*<0.05) of UVC irradiation on the carbohydrate content of peanuts at both studied distances,15 and 30 cm, as it recorded 15.92% in samples without irradiation to reach values of 14.98% and 15.08%, respectively after 12 h of treatment. Wang et al. [40] showed that wheat grains treatment with 75 mg/l ozone for up to 90 min did not cause significant differences in starch content of ozone-treated samples.

Insignificant differences (P<0.05) in feed ash content after UVC+O, treatment were observed in our study. These results were in agreement with the results presented by Sobeli et al. [39] which exhibited an insignificant effect (P > 0.05) of pulsed UVC irradiation on the ash content of beef loin steaks. Also, an insignificant change (P < 0.05) in the ash content of maize after treatment with 13.5 mg/l ozone for up to 60 h was recorded by Ribeiro et al. [17]. Results exhibited a significant decrease (P<0.05) in feed ash content only at a 15 cm distance after 180 min of UVC+O₃ exposure. A decrease in the ash content of broiler feeds after ozone treatment at different concentrations (0.9 and 5.6 g/h) and time durations (15 and 30 min) was demonstrated by Celik et al. [41].

The fats and oils are classified in accordance to their oxidative quality into four categories depending on PVs estimation. If the PVs of fats less than 5 meq/kg indicate the absence of oxidation, PVs between 5-10 meg/kg refer to the appearance of the first signs of oxidation, PVs between 10-20 meg/kg indicate the presence of oxidation, while PVs more than 20 meg/kg refer to the presence of a strong oxidation [42]. Our results showed a significant increase (P<0.05) in PVs after UVC+O3 treatment, but values still less than 5 meq/kg, pointed out that poultry feed fats were still fresh and having good quality. No studies referred to the synergistic effect of UVC+O₂ treatment of poultry feeds on their fat content, however, one study clarified their synergistic effect on PVs of peanut oil, which were not significantly affected after combined treatment for 30 min [20]. The effect of UVC or O₃ treatment on the fat content and PVs in many food commodities was investigated by many research

studies. Results were in agreement with the results obtained by Garg et al. [37] which recorded a decrease in the peanut fat content from 48.32% to 44.50% and 44.76% after UVC irradiation at distances of 15 and 30 cm, respectively for 12 h. While PVs of peanut oil were slightly affected as it recorded 9.19 meq/kg in untreated samples to amount values of 9.24 and 9.17 meg/kg in the samples irradiated at 15 and 30 cm distances, respectively. Perna et al. [43] demonstrated that ozonation of cream with an inlet flow of 300 mg O₃/h significantly increases PV (P<0.05), in a time-dependent manner, as it increases from 0.288 meg/kg in untreated samples to reach 4.086 meg/ kg after 60 min of treatment. The decline in fat content with the increment in PVs may return to fat oxidation by UVC and O, [43, 44]. Our results showed insignificant differences (P<0.05) in the fat content of feeds treated with UVC+O, for 180 min at the third distance only (60 cm). Sobeli et al. [39] exhibited an insignificant effect (P > 0.05)of pulsed UVC radiation on the total fat content of beef loin steak samples. Also, an insignificant change (P < 0.05) in the fat content of maize was recorded after treatment with 13.5 mg/l ozone for up to 60 h [17].

Conclusions

The present study showed the efficiency of UVC+O₃ as a promising non-thermal technology for AFB1, OTA, and FB1 detoxification from naturally contaminated poultry feeds. Mycotoxins degradation increased with increasing the exposure time and decreasing the distances. UVC+O₃ treatment for 10 min was sufficient to reach to AFB1 legal limit at the three examined distances and 20 min to reach the OTA legal limit at 15 and 30 cm. The degradation levels of OTA and FB1 were significantly higher (*P*<0.05) than those recorded for AFB1. While the feed components were moderately affected, the feed fats remained fresh and of good quality after UVC+O₃ application.

Conflict of interest

The authors declare that there is no conflict of interest.

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Author's contributions

Hiba Salahaldeen Alnaemi proposed the study idea. All authors contributed to the study design. Hiba Salahaldeen Alnaemi performed the experiments. Qais Thanon Algwari supervised the UVC+O₃ experiments. Hiba Salahaldeen Alnaemi, Tamara Natiq Dawood and Qais Thanon Algwari analyzed the data. Hiba Salahaldeen Alnaemi wrote the first draft of the manuscript. Tamara Natiq Dawood and Qais Thanon Algwari supervised and revised the manuscript writing. All the authors reviewed and approved the final version of the manuscript.

TABLE 1. UVC doses at different distances from an ozone-generating UVC lamp

TD' (')		UVC dose (mJ/cm²)	
Time (min)	15 cm	30 cm	60 cm
10	577.8	397.8	50.4
20	1155.6	795.6	100.8
30	1733.4	1193.4	151.2
60	3466.8	2386.8	302.4
120	6933.6	4773.6	604.8
180	10400.4	7160.4	907.2

Data are expressed as means of three replicates.

TABLE 2. Effect of UVC+O₃ treatment at 15 cm on AFB1, OTA and FB1 mean concentrations and degradation percentages in poultry feeds

Treatment	AFB1		OTA		FB1		
time (min) Conc. (µg/kg)	Deg. (%)	Conc. (μg/kg)	Deg. (%)	Conc. (µg/kg)	Deg. (%)	Time effect	
0	23.71	0 a	164.66	0 a	6850.25	0 a	0 A
10	14.47	38.97 bc	104.99	35.96 b	3932.04	42.60 c	39.18 B
20	12.67	46.57 de	89.85	45.29 d	3425.13	50.00 e	47.28 C
30	11.07	53.24 f	71.92	56.18 f	2933.28	57.18 f	55.53 D
60	8.1	65.73 g	43.29	73.60 h	1972.87	71.20 h	70.18 E
120	6.61	72.08 i	31.43	80.87 j	1438.55	79.00 j	77.32 F
180	4.52	80.94 k	26.13	84.07 jk	1123.44	83.60 k	82.87 G
Mycotoxin effect		51.07 A		53.71 B		54.8 B	

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P < 0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).
- Concentration (Conc.), Degradation (Deg.).

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TABLE 3. Effect of UVC+O₃ treatment at 30 cm on AFB1, OTA and FB1 mean concentrations and degradation percentages in poultry feeds

Treatment time	AI	FB1	O	ГА	FB	1	
(min)	Conc. (µg/kg)	Deg. (%)	Conc. (µg/kg)	Deg. (%)	Conc. (µg/kg)	Deg. (%)	Time effect
0	23.71	0 a	164.66	0 a	6850.25	0 a	0 A
10	15.09	36.34 b	104.86	36.11 b	4164.95	39.20 b	37.21 B
20	13.55	42.85 ^C	87.68	46.60 ^d	3706.67	45.89 ^{cd}	45.11 ^c
30	11.81	50.19 e	73.69	55.15 f	2951.09	$56.92~^{\rm f}$	54.08 ^D
60	8.42	64.49 ^G	49.94	69.50 h	2178.38	68.20 h	67.4 ^E
120	6.16	74.01^{i}	34.04	79.31 ^j	1703.66	75.13 ⁱ	76.15 ^F
180	5.1	78.49 k	26.39	83.89 1	1103.58	83.89 1	82.09 ^G
Mycotoxin effect		49.48 ^A		52.94 ^B		52.75 ^B	

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- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 4. Effect of UVC+O₃ treatment at 60 cm on AFB1, OTA and FB1 mean concentrations and degradation percentages in poultry feeds

Treatment time	AF	B1	07	ГА	F	B1	
(min)	Conc.	Deg. (%)	Conc. (µg/kg)	Deg. (%)	Conc. (µg/kg)	Deg. (%)	Time effect
0	23.71	0 a	164.66	0 a	6850.25	0 a	0 A
10	18.98	19.94 ^b	127.33	22.38 b	5372.65	21.57 b	21.3 B
20	17.13	27.75 °	110.8	32.49 d	4638.30	32.29 d	30.84 ^c
30	14.06	40.70 °	89.59	45.34 f	3598.44	47.47 f	44.50 D
60	11.37	52.03 g	73.47	55.23 g	3091.52	54.87 ^g	54.05 E
120	8.65	63.51 h	53.02	67.69 i	2500.34	63.50 h	64.90 F
180	7.61	67.90^{j}	41.46	74.76 k	1857.1	72.89 k	71.85 ^G
Mycotoxin effect		38.83 A		42.56 B		41.8 ^B	

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- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).
- Concentration (Conc.), Degradation (Deg.).

TABLE 5. Effect of UVC+O₃ treatment at different distances and time durations on AFB1 degradation levels in poultry feeds

Tuesday and times (min)		Degradation (%)					
Treatment time (min) —	15 cm	30 cm	60 cm	Time effect			
0	0 a	0 a	0 a	0 A			
10	38.97 b	36.34 b	19.94 °	31.75 ^B			
20	46.57 ^d	42.85 ^d	27.75 °	39.05 ^c			
30	53.24 ^f	50.19 f	40.70 g	48.04 ^D			
60	65.73 h	64.49 h	52.03 i	60.75 E			
120	72.08^{j}	74.01 ^j	63.51 k	69.87 ^F			
180	80.94 1	78.49 ¹	67.90 m	75.78 ^G			
Distance effect	51.07 A	49.48 B	38.83 ^c				

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 6. Effect of UVC+O₃ treatment at different distances and time durations on OTA degradation levels in poultry feeds

[Degradation (%)					
Treatment time (min) —	15 cm	30 cm	60 cm	Time effect			
0	O ^a	O ^a	O ^a	0 ^A			
10	35.96 ^b	36.11 ^b	22.38°	31.48^{B}			
20	45.29 ^d	46.60^{d}	32.49e	41.46 ^C			
30	56.18 ^f	55.15 ^f	45.34 ^g	52.22 ^D			
60	73.60^{h}	69.50^{h}	55.23i	66.11 ^E			
120	80.87^{j}	79.31^{j}	67.69 ^k	75.96^{F}			
180	84.07 ^j	83.89 ^j	74.76^{1}	80.91^{G}			
Distance effect	53.71 ^A	52.94 ^A	42.56^{B}				

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P < 0.05).

TABLE 7. Effect of UVC+O₃ treatment at different distances and time durations on FB1 degradation levels in poultry feeds.

Tuestan and times (min)	Degradation (%)					
Treatment time (min) —	15 cm	30 cm	60 cm	Time effect		
0	O ^a	O ^a	0a	0 ^A		
10	42.60 ^b	39.20°	21.57 ^d	34.46^{B}		
20	50.00e	$45.89^{\rm f}$	32.29^{g}	42.73°		
30	57.18 ^h	56.92 ^h	47.47^{i}	53.86 ^D		
60	71.20^{j}	68.20k	54.871	64.76 ^E		
120	$79.00^{\rm m}$	75.13 ⁿ	63.50°	$72.54^{\rm F}$		
180	83.60 ^p	83.89^{q}	72.89^{r}	80.13 ^G		
Distance effect	54.8 ^A	52.75 ^B	41.8 ^c			

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P < 0.05).
- Horizontally different small letters are significantly different at (P < 0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 8. HPLC analysis of poultry feed samples showed highest co-occurrence and highest degradation levels of AFB1, OTA and FB1 after UVC+O₃ treatment at 15 cm.

Treatment time	AFB1		ОТ	A	FB1	
(min)	Conc. µg/kg	Deg. %	Conc. μg/kg	Deg. %	Conc. µg/kg	Deg. %
0	21.84	0	149	0	6613.5	0
120	5.64	74.18	25.22	83.07	1359.54	79.44
180	3.67	83.20	18.89	87.32	979.25	85.19

- Data are expressed as means of three replicates.
- Concentration (Conc.), Degradation (Deg.).

TABLE 9. HPLC analysis of poultry feed samples showed highest co-occurrence and highest degradation levels of AFB1, OTA and FB1 after UVC+O₃ treatment at 30 cm

Treatment time	AFB1		OT	A	FB1	
(min)	Conc. µg/kg	Deg. %	Conc. µg/kg	Deg. %	Conc. µg/kg	Deg. %
0	21.84	0	149	0	6613.5	0
120	5.37	75.41	29.83	79.98	1618.41	75.53
180	3.92	82.05	22.07	85.19	950.11	85.63

- Data are expressed as means of three replicates.
- Concentration (Conc.), Degradation (Deg.).

TABLE 10. Effect of UVC+O₃ treatment on the moisture content of poultry feeds

		Moisture content (%)					
Treatment time (min)	15 cm	30 cm	60 cm	Time effect			
0	9.85ª	9.85ª	9.85ª	9.85 ^A			
10	9.55 ^b	9.59 ^b	9.62 ^b	$9.59^{\rm B}$			
20	9.23°	9.31°	9.42 ^d	9.32 ^c			
30	8.89°	$9^{\rm f}$	9.17 ^g	9.02^{D}			
60	8.47 ^h	8.57 ⁱ	8.8^{j}	8.61 ^E			
120	7.97^{k}	8.08^{1}	8.39 ^m	8.15^{F}			
180	7.52 ⁿ	7.73°	$8.07^{\rm p}$	7.77^{G}			
Distance effect	8.78^{A}	8.88^{B}	9.05°				

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 11. Effect of UVC+O₃ treatment on the protein content of poultry feeds.

	Protein content (%)						
Treatment time (min)	15 cm	30 cm	60 cm	Time effect			
0	20.88ª	20.88ª	20.88ª	20.88 ^A			
10	20.82 ^b	20.86^{ab}	20.87 ^a	20.85^{B}			
20	20.84^{ab}	20.83 ^{bc}	20.87^{ac}	20.85^{B}			
30	20.76°	20.85^{ab}	20.84^{ag}	20.82 ^c			
60	20.64^{de}	20.79°	20.86^{ab}	20.76^{D}			
120	20.68^{d}	20.72^{d}	20.82^{bg}	20.74^{D}			
180	20.63°	20.69 ^d	20.81 ^g	$20.71^{\rm E}$			
Distance effect	20.75 ^A	20.80^{B}	20.85°				

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 12. Effect of UVC+O ₃	treatment on the carbohydrate conte	nt of poultry feeds

Treatment time (min)	Carbohydrate content (%)			
	15 cm	30 cm	60 cm	Time effect
0	56.16ab	56.16ab	56.16a	56.16 ^{AC}
10	56.14a	56.16 ^{ab}	56.15a	56.15^{AB}
20	56.15a	56.15 ^{ab}	56.13a	56.14 ^A
30	56.14a	56.14a	56.14a	56.14 ^A
60	56.16 ^{ab}	56.15 ^{ab}	56.13a	56.15 ^A
120	56.17 ^{ab}	56.19 ^b	56.16ab	56.17^{BC}
180	56.2 ^b	56.18ab	56.17 ^{ab}	56.18
Distance effect	56.16 ^A	56.16 ^A	56.15 ^A	

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 13. Effect of UVC+O, treatment on the ash content of poultry feeds

Treatment time (min)	Ash content (%)			
	15 cm	30 cm	60 cm	Time effect
0	9.28a	9.28a	9.28a	9.28 ^A
10	9.27a	9.3ª	9.28ª	9.28^{A}
20	9.28a	9.28^{a}	9.29a	9.28^{A}
30	9.29a	9.27ª	9.28ª	9.28^{A}
60	9.27a	9.24a	9.27ª	9.26^{AC}
120	9.25a	9.26a	9.23a	9.25^{BC}
180	9.19 ^b	9.25ª	9.24^{ab}	9.23^{B}
Distance effect	9.26^{A}	9.27^{A}	9.27^{A}	

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 14. Effect of $UVC+O_3$ treatment on the fat content of poultry feeds

Treatment time (min) —	Fat content (%)			
Treatment time (mm)	15 cm	30 cm	60 cm	Time effect
0	2.89a	2.89a	2.89a	2.89 ^A
10	2.85^{ab}	2.87^{ac}	2.88a	2.87^{AB}
20	2.88^{a}	2.86^{ac}	2.89a	2.88^{A}
30	$2.81^{\rm bf}$	2.87^{ac}	2.86^{a}	2.85^{B}
60	2.73°	2.83^{bc}	2.86^{ab}	2.81 ^c
120	2.78^{df}	2.77^{d}	2.85a	2.8°
180	2.75^{de}	2.79^{bd}	2.87^{a}	2.80°
Distance effect	2.81 ^A	2.84^{B}	2.87°	

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (P<0.05).
- Horizontally different small letters are significantly different at (P < 0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

TABLE 15. Effect of UVC+O₃ treatment on the PVs of poultry feed fats

Treatment time (min)	PVs (meq/kg)			
	15 cm	30 cm	60 cm	Time effect
0	3.85a	3.85a	3.85a	3.85 ^A
10	3.88a	3.87a	3.86a	3.87^{AB}
20	3.94 ^b	3.87^{a}	3.85^{a}	3.89^{B}
30	4.04°	3.86^{a}	3.87a	3.92 ^c
60	4.13 ^d	3.92^{b}	3.88^{ab}	3.98^{D}
120	4.2e	4.08^{g}	3.92^{bc}	4.07^{E}
180	$4.25^{\rm f}$	4.13^{d}	3.95°	4.11^{F}
Distance effect	4.04^{A}	3.94^{B}	3.88 ^c	

- Data are expressed as means of three replicates.
- Vertically different small letters are significantly different at (*P*<0.05).
- Horizontally different small letters are significantly different at (P<0.05).
- Different capital letters within last row and column are significantly different (P<0.05).

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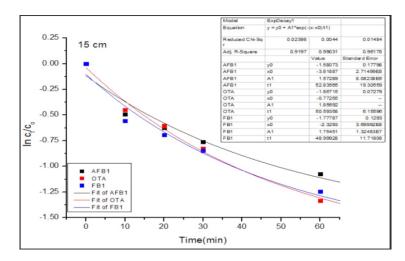


Fig. 1. Degradation kinetics of AFB1, OTA and FB1 in poultry feeds after UVC+O₃ application at 15 cm for different time durations.

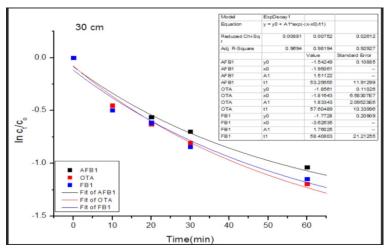


Fig. 2. Degradation kinetics of AFB1, OTA and FB1 in poultry feeds after UVC+O3 application at 30 cm for different time durations.

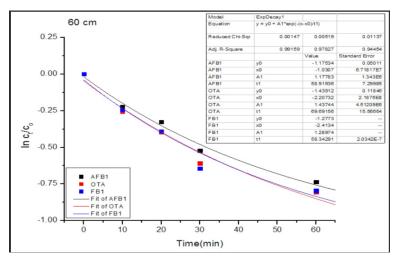


Fig. 3. Degradation kinetics of AFB1, OTA and FB1 in poultry feeds after UVC+O₃ application at 60 cm for different time durations.

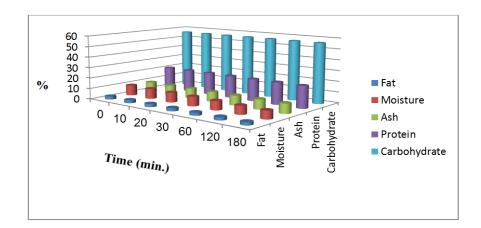


Fig. 4. Effect of UVC+O3 treatment at 15 cm on the nutritional composition (%) of poultry feeds.

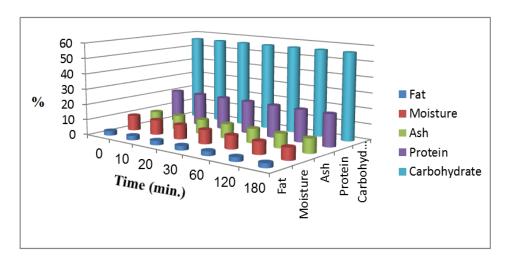


Fig. 5. Effect of UVC+O3 treatment at 30 cm on the nutritional composition (%) of poultry feeds.

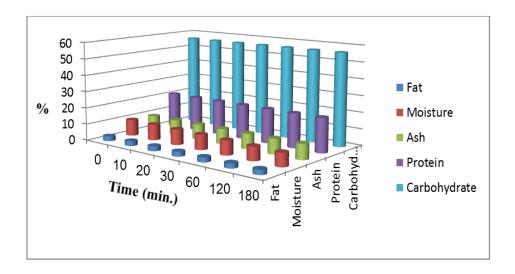


Fig. 6. Effect of UVC+O3 treatment at 60 cm on the nutritional composition (%) of poultry feeds.

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A المتخدام الأشعة فوق البنفسجية C والأوزون لإزالة سمية سم الأفلا B1 وسم الاوكرا وسم الفومونيزين B1 في أعلاف الدواجن

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والأوزون في ازالة سمية كل من C تضمنت الدراسة التحري عن كفاءة التأثير التآزري للأشعة فوق البنفسجية في أعلاف الدواجن الملوثة طبيعياً مع دراسة تأثيره على B1 وسم الفومونيزين A وسم الاوكرا B1 سم الأفلا والأوزون لست فترات زمنية C المكونات الغذائية للأعلاف. تم تعريض عينات العلف للأشعة فوق البنفسجية (10، 20، 30، 60، 120، و 180 دقيقة) على بعد 15 و 30 و 60 سم من مصدر الأشعة فوق البنفسجية وبجرع تر اوحت مابين 577.8 إلى 10400.4 مللي جول/ سم² عند 15 سم، و397.8 إلى 7160.4 مللي جول/ سم² عند 30 سم، و50.4 إلى 907.2 مللي جول/ سم 2 عند 60 سم عند تركيز اوزون ثابت قدره 10 جزء في المليون. تم وتم تأكيد النتائج (ELISA) تقدير مستويات السموم الفطرية بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم تم تحليل مكونات العلف وتقدير قيم البيروكسيد وفقًا (HPLC) بواسطة تقنية الاستشراب السائل عالى الأداء B1 في مستويات تحلل كل من سم الأفلا (P<0.05) للطرق التحليلية القياسية. سجلت النتائج ارتفاعا معنويا مع زيادة زمن التعرض وتقليل المسافات لتصل إلى قيم 80.94 وB1 84.07 وسم الفومونيزين A وسم الاوكرا و83.6% عند 15 سم و78.49 و83.89 و83.89 عند 30 سم و67.9 و 74.76 و72.89% عند 60 سم، مستويات تدهور B1 وسم الفومونيزين A على التوالي بعد 180 دقيقة من المعاملة. أظهر كل من سم الاوكرا تأثرت المكونات العلفية بدرجة متوسطة بالمعاملة .B1 من تلك المسجلة لسم الأفلا (P<0.05) أعلى معنويا والأوزون. أظهرت النتائج أن دهون العلف لا تزال تتمتع بنوعية جيدة اعتمادا على C بالأشعة فوق البنفسجية B1 والأوزون في ازالة سمية سم الأفلا C تقدير قيم البيروكسيد. كفائة التأثير التآزري للأشعة فوق البنفسجية من اعلاف الدواجن مع تأثير متوسط على جودة العلف B1 وسم الفومونيزين A وسم الاوكرا

الكلمات الدالة: جودة العلف، سموم فطرية، اوزون، الأشعة فوق البنفسجية C.