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Use of gaseous ozone to reduce aflatoxin B_1 and microorganisms in poultry feed

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ABSTRACT

This study was undertaken to evaluate the efficacy of gaseous ozone for the degradation of aflatoxin B_1 (AFB_1) and inactivation of indigenous microflora in poultry feed. Feed samples were treated with continuous stream of two different constant concentrations (2.8 and 5.3 mg/L) of ozone at room temperature up to 240 min. The initial AFB_1 level in artificially contaminated feed samples, determined as 32.8 μg/kg, decreased by 74.3 and 86.4% after 240 min of exposure at 2.8 and 5.3 mg/L, respectively. At the both ozone concentrations, 240 min exposure was reduced the aerobic plate and yeast and mold counts below the detection limit (<10 CFU/g) with a reduction more than 3.2 and 2.7 log, respectively. The thiobarbituric acid reactive substances (TBARS) assay indicated that no significant ($P \geq 0.05$) increase occurred in the level of lipid oxidation in feed samples during 120 min ozonation at 2.8 mg/L. At the end of the 240 min of exposure at 2.8 and 5.3 mg/L, initial TBARS concentration, determined as 2.4 mg/kg, reached to 4.4 and 5.3 mg/kg with a significant ($P < 0.05$) increases, respectively. The results presented in this study suggested that significant ($P < 0.05$) reductions in the AFB_1 level and microbial population can be achieved in poultry feed by ozonation with an acceptable changes in lipid oxidation.

1. Introduction

Feed materials may be contaminated with bacteria and fungi at any time during growing, harvesting, processing and storage. Therefore, animal feeds may serve as a potential reservoir for a wide variety of microorganisms including animal pathogens. Microbial contamination can also affect feed quality negatively in numerous ways including reducing dry matter and nutrients, causing musty or sour odours and causing caking of the feed (Maciorowski et al., 2007). In addition, some species of filamentous fungi can produce mycotoxins under certain conditions such as elevated temperature and relative humidity (Karaca et al., 2010).

Aflatoxins (AFs), a group of toxic secondary metabolites produced by certain species of *Aspergillus*, are regarded as the most prevalent mycotoxins in animal feeds. AFs affect all classes of livestock, although a wide variation exists in species susceptibility to AFs (Rawal et al., 2010). Poultry are included among the most susceptible animal species to AFs (Dalvi, 1986). Even when exposure does not cause mortality or morbidity, aflatoxicosis contributes directly and indirectly to losses for the poultry industry. Of the known AFs, aflatoxin B_1 (AFB_1) is considered as the most biologically active and toxic form. AFB_1 is associated with hepatotoxicity, carcinogenicity, genotoxicity, immunosuppression and other deleterious effects in many animal species including poultry (Richard, 2007; Rawal et al., 2010; Kalpana et al., 2015). There are numerous reports on the profound and negative impact of feed-borne AFB_1 contamination on feed efficiency, which significantly reduces productivity in the poultry industry (Rawal et al., 2010; Yunus et al., 2011; Monson et al., 2015). Furthermore, AFB_1 can pass to the by-products of poultry such as egg and meat, therefore posing a potential threat to human health (Trucksess and Stoloff, 1981; Trucksess et al., 1983).

Exposure of poultry to AFB_1 primarily occurs by consumption of contaminated corn, grain or other feed components. Despite improvements in biological methods, handling, processing and storage, AFB_1 contamination still remains a serious problem for the animal feed industry and an ongoing risk to the security of the feed supply (Bryden, 2012). A variety of chemical treatments have been studied in connection with their effectiveness to degrade AFB_1 and

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eliminate fungal viability in feedstuffs (Méndez-Albores et al., 2007). However, most of these chemicals employed have carcinogenic and teratogenic attributes as well as residual toxicity (Xiong, 2012; Monson et al., 2015).

Ozone is a powerful and environment-friendly antimicrobial substance due to its potential oxidizing capacity. Apart from the wide spectrum of microbial inactivation, ozone also has the potential to degrade mycotoxins (Tiwari et al., 2010). Because ozone requires no storage or special handling or mixing considerations, it may be viewed as advantageous compared to other chemical sanitizers (Guzel-Seydim et al., 2004). It is convenient for the decontamination of agricultural commodities, as it quickly decomposes into oxygen and hence does not leave undesirable by-products or residues (Graham, 1997).

Management of AFs and microbial contamination in the feed industry includes prevention, regulation and decontamination (Womack et al., 2014). The AF degradation and microbial inactivation methods must ensure that process retains the nutritional, sensory and functional properties of the product (Herzallah et al., 2008). Antimicrobials in the gaseous state are less likely to modify the composition of feed matrices compared to their aqueous solutions and have the advantage of being simple, dry and non-destructive (Perry and Yousef, 2011). Therefore, the present study was conducted to evaluate the efficacy of gaseous ozone treatment for the reduction of microorganisms and AFB_1 levels in poultry feed. We also evaluated the changes in lipid oxidation levels of feed samples during ozonation.

2. Materials and methods

2.1. Feed sample

A commercial poultry feed sample composed of corn (48.3%), soybean meal (21.0%), barley (13.0%), sunflower meal (7.3%), limestone (8.6%), dicalcium phosphate (1.2%), salt (0.3%), vitamin/mineral pre-mix (0.3%) and methionine (0.2%) was kindly provided by Intravet Animal Feeding Company (Konya, Turkey). It was verified as free from AFB_1 (<0.1 μg/kg) by an official food control laboratory (Konya, Turkey) which was accredited for mycotoxin analysis of feeds according to ISO/IEC 17025 standard. Feed sample was first divided into two sub-samples and one of them was contaminated artificially with AFB_1. Effect of ozonation on microbial quality and lipid oxidation was evaluated on non-contaminated sub-sample.

2.2. AFB_1 contamination

A methanolic stock solution of AFB_1 at a concentration of 20 μg/mL (Supelco, Bellefonte, PA, USA) was diluted five-fold in methanol to produce the working solution. Five hundred grams of feed sub-sample allocated to the AFB_1 contamination was spread on an aluminum tray. Then, 5 mL of the working solution of AFB_1 was sprayed as homogeneously as possible on the feed sample using an atomizer (DeVilbiss Healthcare, Somerset, PA, USA). After drying for 30 min, the contaminated sample was transferred to a stomacher bag (Gosselin, Hazebrouck Cedex, France) and thoroughly mixed by hand for 5 min. Thus, 40 μg/kg contamination level of AFB_1 was achieved in the sub-sample.

2.3. Ozone treatment

The sub-samples of poultry feed were divided into portions of 50 g in petri dishes (150 × 25 mm) and subjected to gaseous ozone at two different constant concentrations for four exposure times (30, 60, 120 and 240 min). A continuous stream of gaseous ozone was delivered to feed portions at room temperature in 9.9 L plexiglas desiccator chambers (Belart Products, Wayne, NJ, USA) equipped with two gas ports for the inlet and outlet flow. Ozone was generated directly from atmospheric oxygen by two generators (Opal, Ankara, Turkey) with different ozone generation capacities. Air flow rates in the tubes connected to inlet ports of chambers were adjusted to 1 L/min using a flow meter (Dwyer Instruments, Michigan City, IN, USA). The ozone concentrations in the air flows were determined as 2.8 and 5.3 mg/L by the iodometric method based on ozone/iodine stoichiometry of 1 (IOA, 1996).

The durations necessary for the ozone concentrations in the treatment chambers to reach asymptotic concentration were calculated by a mass balance equation previously described by Silva et al. (1998):

$$C_0 \times \left(1 - e^{\left(-v \times \frac{t}{V_t}\right)}\right) = C$$

where V_t is the volume of chamber (L), v is the air flow (L/min), t is the time (min), C_0 is the concentration of ozone coming from generator (mg/L) and C is the predicted ozone concentration in the chamber for specified time (mg/L).

2.4. AFB_1 analysis

Ozonated portions of the feed sub-sample spiked with AFB_1 were assayed in order to quantitate the AFB_1 using a commercial ELISA kit (Romer Labs, Tull, Austria). The portions were ground using a laboratory mill (Ika, Staufen, Germany) so that 95% would pass through a 20-mesh screen. Twenty grams of ground samples were weighed into flasks and 100 mL extraction solvent (Methanol-water, 70:30, v/v) was added to each flask. Then, the mixtures were homogenized with a shaker (Nüve, Ankara, Turkey) for 3 min and allowed to settle for 5 min. The top layers of extracts were filtered through filter paper (Whatman No. 1, 27 cm diameter) and filtrates were used in ELISA assay according to manufacturer instruction. The microwells were measured optically using an ELISA reader (Thermo, Waltham, MA, USA) with an absorbance filter of 450 nm and a differential filter of 630 nm. The amounts of AFB_1 in the sample extracts were quantified by a calibration curve constructed with the standard solutions of AFB_1. Finally, AFB_1 concentrations in the original samples were calculated as μg/kg. The quantitation range of assay was 2–50 μg/kg. The mean recovery and relative standard deviation of repeatability evaluated by the multiple analyses of a spiked feed sample were 82.5% and 7.4%, respectively.

2.5. Microbiological analysis

Microbiological enumerations were performed by a plate count technique on plate count agar (PCA, Lab M, Bury, UK) and dichloran rose bengal chloramphenicol (**DRBC**) **agar** (Lab M) for aerobic plate (AP) and yeast and mold (YM) counts, respectively. Initial suspensions were prepared with adding 90 mL buffered peptone water (Lab M) into stomacher bags containing a 10 g sample. Also, 1 mL of initial suspension and additional ten-fold dilutions were surface plated on three plates of enumeration media. Inoculated PCA plates were incubated at 35 °C for 48 h for AP counts, while DRBC agar plates were incubated at 25 °C for 5 days for YM counts. After incubation all colonies grown on PCA and DRBC agar plates were counted and microorganism counts were calculated as log CFU/g.

2.6. Determination of lipid oxidation

Lipid oxidation levels in feed portions before and after ozonation were measured by the thiobarbituric acid reactive substances