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Medically important polycyclic aromatic hydrocarbon levels in char grilled and barbecued beef, fish, and poultry meat sold in Al-Qalyubia governorate.

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ABSTRACT

This study aimed to quantify and compare polycyclic aromatic hydrocarbons (PAHs) levels in char grilled and barbecued beef, fish, and poultry meat samples (21) collected from Al-Qalyubia governorate, Egypt, by the most contemporary analytical technology, gas chromatography-mass spectrometry. The pH did not differ amongst the various meat categories ($P > 0.05$). The levels of B[a]P and PAH4, and probably- (D[a,h]A) and possibly-carcinogenic PAHs levels in the various meat categories did not differ statistically ($P > 0.05$). Statistically, chicken meat had the lowest mean (0.005 µg/kg) of B[a]P compared to fish (0.232 µg/kg) and beef (0.729 µg/kg). B[a]P ranges were 0.033-0.522 µg/kg in fish, 0.002-4.791 µg/kg in beef, and 0.003-0.008 µg/kg in poultry. One beef sample (4.79 µg/kg) exceeded the B[a]P optimum authorized limit of 2 µg/kg. This sample also contained the highest possibly-carcinogenic PAHs concentrations (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-c, d] pyrene. The PAH4 ranges varied from 0.082 to 0.587 µg/kg in fish, 0.039 to 7.870 µg/kg in beef, and 0.038 to 0.049 µg/kg in poultry. Fish had a greater mean PAH4 level (0.308 µg/kg) compared to poultry (0.041 µg/kg) ($P < 0.05$). The PAH4 mean was highest in beef (1.217 µg/kg), however it was statistically comparable to other meat ($P > 0.05$). 96.67% of the samples were safe, according to the safety requirements. Future comprehensive studies that evaluate the margin of exposure along with potential cancer risk from consuming different types of meat should be carried out to predict potential health issues in the Egyptian population.

1. INTRODUCTION

PAHs are a group of approximately 200 persistent organic compounds with a chemical makeup that includes two or more fused benzene rings. They are produced through the pyrolysis of organic molecules or incomplete industrial combustion (Abbas et al., 2018; Purcaro et al., 2013).

Oils, fats, meat, fruit, vegetables, dairy products, cereals, smoked and unsmoked meat products, and condiments (spices) are the most prevalent sources of PAHs. Yet, much research on PAHs shows that meat products are among the foods that contain the greatest levels of PAHs (Chiang et al., 2021; Martorell et al., 2010).

Crucially, PAHs have several detrimental consequences on human health, primarily linked to immunosuppressive effects as well as carcinogenesis and mutagenesis. Even while not all PAHs are considered carcinogens, their combined effects on human health can still be detrimental due to their position as free radicals and their ability to bioaccumulate and cause cellular damage (Sampaio et al., 2021). PAHs' impacts on human health are determined by various parameters, including exposure duration and route, concentration, and toxicity (International Agency for Research on Cancer, 2010; Sampaio et al., 2021). Most studies link PAHs to carcinogenesis, and recent research suggests that frequent exposure may increase the risk of oxidative stress, thrombosis, hypertension, myocardial infarction, and cardiovascular diseases (Mallah et al., 2022; Sampaio et al., 2021; Sun et al., 2019).

The sources of PAHs in meat are meat processing techniques, including smoking and drying (Shamloo et al., 2024). The primary source of PAHs is smoking, which can be classified as either direct or indirect (Codex Alimentarius Commission, 2009; Ledesma et al., 2016). Direct procedures, also known as traditional techniques, involve smoking the meat in the same chamber where combustion occurs (Codex Alimentarius Commission, 2009; Ledesma et al., 2016). Thus, inefficient fuel combustion is the main cause of PAH accumulation on the food surface (Codex Alimentarius Commission, 2009; Ledesma et al., 2016). On the other hand, liquid smoke or smoke from a friction generator is used in the indirect approach (Codex Alimentarius Commission, 2009; Ledesma et al., 2016).

The European Food Safety Authority (EFSA), Committee for Food Additives (JECFA), and Scientific Committee for Food (SCF) have established that sixteen PAHs may be detected in food (EFSA, 2008). Carcinogenic substances were divided into four groups by the International Agency for Research on Cancer (IARC): Group 1 (carcinogenic to humans), Group 2A (probably carcinogenic to humans), Group 2B (possibly carcinogenic to humans), and Group 3 (not classifiable as to its carcinogenicity to humans for now). Benzo[a]pyrene (BaP) is a member of Group 1 and has been the subject of decades of research because of its harmful effects, especially carcinogenicity and genotoxicity (Dong et al., 2023). The chemical dibenz[a,h]anthracene (D(a,h)A) is in Group 2A. Other chemicals in Group 2B are benz[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B(k)F), and indeno [1,2,3-c, d] pyrene (I(c,d)P) (IARC, 2010).

In 2008, The European Food Safety Authority's (EFSA) CONTAM Panel determined that B[a]A analysis alone was not an adequate indicator for all genotoxic and carcinogenic PAHs detected in foods (EFSA, 2008). Consequently, the

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EFSA CONTAM Panel recommended examining three more PAHs in conjunction with BaP, making a total of four PAHs (PAH4): B[a]A, Chr, B[b]F, and B[a]A. Comparable levels of PAH4 to eight PAHs (PAH8), including PAH4, B(k)F, I(c,d)P, D(a,h)A, and benzo[g,h,i]perylene (B(g,h,i)P), supported the EFSA CONTAM Panel's opinion that PAH4 is a good indicator of the presence of PAH8 compounds in food (EFSA, 2008). Besides, the European Commission created a distinct maximum level for B[a]P to facilitate future data comparison (EFSA, 2008). Regulation No. 835/2011 established the maximum concentrations of B[a]P and PAH4 in smoked meat at 2 and 12 µg/kg, respectively (EU, 2011).

In Egypt, NFSA adopted the same acceptable limits of EFSA for B[a]P and PAHs4 (B[a]A, Chr, B[a]P, B[b]F) in Egyptian meat and meat products by no more than 2 and 12 µg/kg, respectively (EFSA, 2008; NFSA, 2022).

It was hypothesized that the levels of PAHs will vary significantly between red meat, white meat, and fish, with grilling and barbecuing contributing to higher concentrations. Therefore, this study aimed to quantify and compare PAH levels generated in char-grilled and barbecued beef, fish, and poultry meat samples sold in Al-Qalyubia governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Experiment management and approval.

The methods employed in this work were authorized by the Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) of Benha University's Faculty of Veterinary Medicine, with the number BUFVM 17-08-2024.

2.2. Sample collection

Twenty-one samples of three different meat categories—fish, beef, and poultry—seven each—were gathered from different restaurants and vendors in Benha and Kafr Shokr, Egypt, and kept at -20 °C until they were analyzed. Following collection of each sample in its serving container, they were all moved to polypropylene containers that had previously been cleaned with methanol .

2.3 pH analysis

A pH-meter analysis using electrodes (Jenway 3510 pH-meter, Cole-Parmer, Staffordshire, United Kingdom) was performed on the samples chosen for pH evaluation after they had each been diluted ten times with sterile distilled water.

2.4 Polycyclic aromatic hydrocarbon (PAH) extraction

After the sample was thawed, a fraction of 100 gm of each frozen sample was used to extract and clean up PAHs in beef, poultry meat and fish. A grinder was used to homogenize each sample after it had been moved to a polypropylene (PP) container. A triplicate aliquot of the homogenized sample was then subjected to alkaline digestion to aid in tissue penetration and subsequent extraction, in which a 1 g aliquot of each sample was combined with 5 mL of a 2 M KOH solution in methanol and agitated on an oscillator shaker for 2 minutes. After that, 10 mL of n-hexane were added to the extracts, and they were sonicated for five minutes in a bath from JP Selecta (Barcelona, Spain) to undergo ultrasound assisted extraction (UAE). After that, the unsaponifiable fat was separated from the organic layer by centrifugation at 5000 rpm (2150 g) for 10 minutes at a low temperature (4°C) on a JP Selecta rotator. After being evaporated under a nitrogen stream, the extract was redissolved in 25 milliliters of an aqueous solution that contained 4% acetonitrile (Rascón et al., 2019). The solid phase extraction (SPE) method used for cleanup the final extract. To condition the SPE columns, 1 mL of acetonitrile, methanol, and filtered water were added sequentially. The organic extract was kept in a 0.5 mL amber glass vial at -18 °C before analysis. Ultimately, the extracts were analyzed by injecting 1 µL aliquots into the GC–MS apparatus (Rascón et al., 2019).

2.5 .Gas chromatography–mass spectrometry (GC-MS) analysis

2.5.1 .GC operating conditions

A Thermo Scientific TRACE GC UltraTM system (Thermo Fisher Scientific, Waltham, MA, USA) was used for the GC analysis. The GC was equipped with a Thermo TR-50MS 30m, I.D. 0.25 mm, 0.25 µm film capillary column. GC conditions included splitless injection mode with a 5mm injection port liner, injection port temperature of 270°C, and flow rate of 1.2mL/min. The split flow setting is set to "On" with a flow rate of 25mL/min and a splitless time of 1min. The SSL carrier method mode was set to constant flow with an initial value of "On", a rate of 1.2mL/min, and an initial time of 1min. The gas saver flow was set at 15mL/min, and the gas saver time was set to 3min. The temperature of the transfer line was 270 ºC, and the vacuum compensation was set to "On". The oven temperature was set to 60 °C for one minute, then programmed at 10 °C/min to 250 °C, then 20 °C/min to 280 °C.

2.5.2. Mass Spectrometric Conditions

A TSQ 8000 evo triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA USA) is used for MS analysis. The mass spectrometer is tuned satisfactorily when the detector is adjusted to m/z 300 or below, the three FC 43 (calibration gas) ions (68, 219, and 502) are at least half the height of their respective windows, and the ions at 502 and 503 are resolved .

The MS conditions for PAHs are listed below: The ionization mode is EI positive ion, the ion volume is closed EI, the emission current is 50uA, and the ion source temperature is 250 °C. The scan type was Full scan in the m/z range 45-650 and SRM, with a scan width of 0.15 for SRM and a scan length of 0.2s for full scan and 0.05 for SRM. Additionally, the peak widths were Q1, 0.7 Da; and Q3, 0.7 Da FWHM. Finally, the collision gas (Ar) pressure reached 0.5 mTorr.

2.5.3 Standard curves

Acetone was used to create a stock solution of each PAH at a concentration of 5 g/L, which was then kept at 4 °C. Every day, stock solutions were converted into working-strength solutions. The eluent, which was made daily in the lab, was acetonitrile with 100 µg/L of the internal standard triphenyl phosphate (Rascón et al., 2019). Figure 1 displays the current estimated standard PAH curves and associated equations.

2.6 . Statistical analysis

The data was analyzed using SPSS Version 22 (SPSS Inc., Chicago, IL, USA). The effects of meat type (fish, poultry, and red meat) on polycyclic aromatic hydrocarbons levels in collected samples were analyzed using a one-way ANOVA. While the muscle was considered random, the animal species were considered fixed variables. The results' means and standard errors are shown. A significant difference was defined as a P value of less than 0.05 (Duncan, 1955).

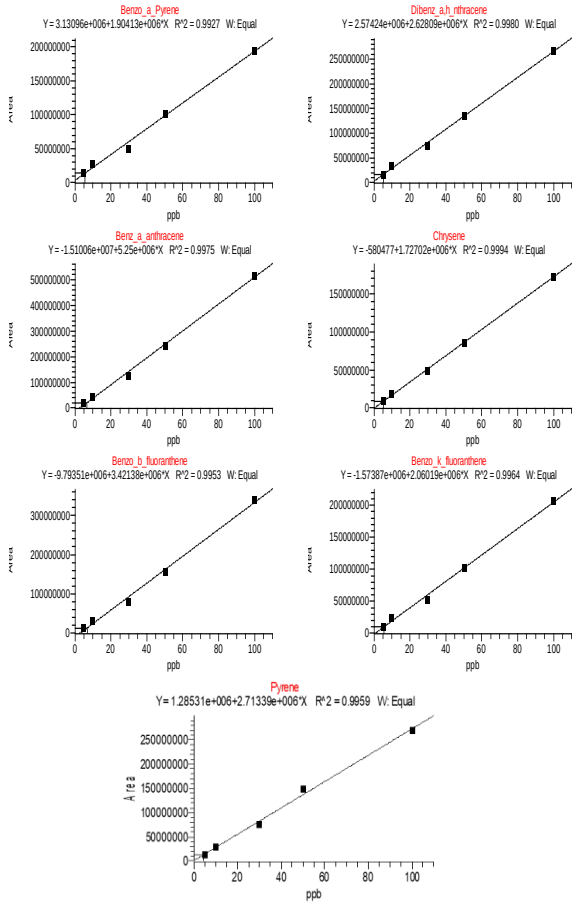

Figure 1 presents the estimated standard PAH curves and corresponding equations. Curves focused on PAHs of public health relevance, benzo[a]pyrene, dibenz[a,h]anthracene chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), and indeno[1,2,3-c,d]pyrene.

3. RESULTS

The pH statistics of the three screened meat categories (fish, beef, and poultry) is shown in Table 1. Statistics indicated that the pH were not different between fish (7.24), beef (7.11), and poultry (7.22) samples (P > 0.05).

Table 1. Compare studied fish, beef, and poultry meat pH.

pHU	Fish	Beef	Poultry	P value
Minimum	6.69	6.6	6.89	0.328
Maximum	7.87	7.86	7.77	
Mean	7.246	7.111	7.221	
Standard Error	0.061	0.080	0.057	

Prevalence of Benzo[a]pyrene (B[a]P) residues in the fish, beef, and poultry meat samples was demonstrated in Table 2. The concentration of B[a]P in different meat categories (fish, beef, and poultry) were not different (P>0.05), however student t. test indicated that chicken meat had the lowest mean concentration (0.005 µg/kg) compared to fish (0.232 µg/kg) and beef (0.729 µg/kg). The range of B[a]P contamination in the meat categories was 0.033-0.522 µg/kg for fish, 0.002-4.791 µg/kg for meat, and 0.003-0.008 µg/kg for poultry (Table 2). In terms of acceptability, remarkably, a beef sample (RM5, 4.79 µg/kg) displayed B[a]P levels considerably greater than the MPL of 2 µg/kg.

Table 2. Prevalence of group 1 classified carcinogenic polycyclic aromatic hydrocarbon residues to humans (benzo[a]pyrene) in the fish, beef, and poultry meat samples.

B[a]P[1] (µg/kg)	Fish	Beef	Poultry	
Minimum	0.033	0.002	0.003	0.428
Maximum	0.522	4.791	0.008	
Mean	0.232	0.729	0.005	
Standard Error	0.059	0.677	0.001	

[1] BaP, benzo[a]pyrene;

Table 3 illustrates the (dibenz[a,h]anthracene, D(ah)A) residues in the three food groups. Fish, beef meat, and poultry all had a comparable concentration of D[a,h]A (P>0.05), but numerically chicken meat had the lower mean concentration (0.0044 µg/kg) in comparison to fish (0.0096 µg/kg) and red meat (2.2477 µg/kg) (P>0.05). Different meat categories had varying levels of D[a,h]A contamination: 0.0001-0.0406 µg/kg for fish, 0.00003-15.61 µg/kg for beef, and 0.0004-0.0105 µg/kg for poultry.

Table 3. Prevalence of Group 2A probably classified carcinogenic Polycyclic aromatic hydrocarbon residues to humans (dibenz[a,h]anthracene) in the fish, beef, and poultry meat samples.

D[a,h]A[1](µg/kg)	Fish	Beef	Poultry	
Minimum	0.0001	0.0000	0.0004	0.383
Maximum	0.0406	15.608	0.0105	
Mean	0.0096	2.2477	0.0044	
Standard Error	0.0054	2.2268	0.0014	

[1]DahA, dibenz[a,h]anthracene;

Table 4 displays the prevalence of group 2B (possibly carcinogenic) Polycyclic aromatic hydrocarbons in humans, including B[a]A, Chr, B[b]F, and B(k)F, in the three food groups. Statistically, there was no discernible variation in the levels of possibly human carcinogenic PAH contamination among the three meat categories (P>0.05). The range of possibly carcinogenic PAHs contamination levels in the various meat categories was 0.058-5.219 µg/kg for fish, 0.040-3.446 µg/kg for beef, and 0.039-0.048 µg/kg for poultry.

Here, acceptable levels of possibly-carcinogenic PAHs to humans in the three meat categories were evaluated using PAH4's maximum permissible limit (MPL) of 12 µg/kg. Here, the sum of the possibly carcinogenic PAH levels across the three meat categories did not exceed 12 µg/kg. Compared to MPL of B[a]P (2 µg/kg), three fish samples (F1, 2.20 µg/kg; F2, 5.11 µg/kg; and F7, 3.20 µg/kg) exhibited B(k)F contamination levels surpassing 2 µg/kg. Additionally, compared to other meat samples, red meat sample number RM5 (3.45µg/kg) had the highest total amounts of possibly carcinogenic PAH pollutants .

Table 4. Prevalence of group 2B classified possibly carcinogenic polycyclic aromatic hydrocarbon residues to humans in the fish, beef, and poultry meat samples.

2B PAH (µg/kg) 1	Fish	Beef	Poultry	
Minimum	0.058	0.040	0.039	0.114
Maximum	5.219	3.446	0.048	
Mean	1.642a	0.598ab	0.041b	
Standard Error	0.761	0.478	0.001	

[1]2B classified Polycyclic aromatic hydrocarbons (possibly carcinogenic to humans, benz[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), and indeno[1,2,3-c,d]pyrene (I[c,d]P))

The prevalence of PAH4, which includes Benz [a] anthracene (B[a]A), chrysene (Chr), benzo [b] fluoranthene (B[b]F), and Benzo[a]pyrene (B[a]P), in the three meat categories is shown in Table 5. The levels of PAH4 contamination in the three types of meat show no statistical difference (P>0.05). Fish had PAH4 levels between 0.082 and 0.587µg/kg, red meat had levels between 0.039 and 7.870 µg/kg, and poultry had levels between 0.038 and 0.049 µg/kg. According to the student T. test, the mean PAH4 level in fish meat was higher (0.308µg/kg) than in poultry (0.041 µg/kg) ($P < 0.05$). Despite having the highest mean (1.217 µg/kg) of any meat category, the T. test indicated that the PAH4 level was similar to that of fish and poultry ($P > 0.05$) .

Table 5. Comparable prevalence of the four polycyclic aromatic hydrocarbons (PAH4) in the fish, beef, and poultry meat samples.

PAH4[1] (µg/kg)	Fish	Beef	Poultry	P value
Minimum	0.082	0.039	0.038	0.415
Maximum	0.587	7.870	0.049	
Mean	0.308^{ab}	1.217^{a}	0.041^{c}	
Standard Error	0.060	1.109	0.002	

[1]Polycyclic aromatic hydrocarbons (PAH4), including Benz [a] anthracene (B[a]A), chrysene (Chr), benzo [b] fluoranthene (B[b]F), and Benzo[a]pyrene (B[a]P).

4. DISCUSSION

Recent research found that traditional processing equipment, such as grilling, smoking, and smoking-drying, contributed significantly to PAHs contamination of meat and fish end products (Assogba et al., 2024). Therefore, this study attempted to assess PAH residue levels in ready-to-eat fish, beef, and poultry meat samples randomly collected from Al-Qalyubia governorate, Egypt.

All the collected samples had a high pH as compared to fresh ones, but there was no species-specific variation. Increased pH during grilling might be caused by a shift in the concentration and makeup of salt, which can alter the pH and alter the reactivity of proteins. The rise in pH during HPP has been linked to the loss of acidic groups in meat because of changes in shape caused by protein denaturation (Şayin Sert and Coşkun, 2022). Similarly, the current study's cooking process relates to protein denaturation, which results in moisture loss and a subsequent increase in solute concentration (Şayin Sert and Coşkun, 2022). Additionally, the currently studied marinated samples might have a higher pH.

The quantity of aromatic rings in a PAH determines its genotoxic characteristics. This kind of activity is shown by four- and five-ring PAHs, with benzo[a]pyrene having the most genotoxic and cancer-causing effects (Myers et al., 2021). The current study indicated that the levels of B[a]P and PAH4, as well as probably D[a, h]A, and possibly carcinogenic PAHs, were not significantly different between meat groups ($P > 0.05$). Chicken meat had the lowest mean of B[a]P compared to fish and beef, according to T-test results. B[a]P levels in one beef sample exceeded the MPL of 2 µg/kg. This sample also has the greatest quantity of possibly carcinogenic PAH contaminants. Fish flesh showed higher mean PAH4 levels compared to chicken ($P<0.05$). While the mean PAH4 level in beef was highest, the T. test demonstrated that it was comparable to fish and poultry ($P > 0.05$).

Prior, comparable research showed that the lowest value of the sixteen PAHs was found in chicken kebab (112.9 µg/kg), and the highest value was found in smoked fish samples (222.7 µg/kg). The samples of grilled chicken and sausage had the lowest mean of 4 PAHs, whereas the highest mean was found in tuna fish (23.7 µg/kg) (Khalili et al., 2023). Their findings demonstrated that the amounts of B[a]P and 4PAHs were below the European Union (EU) standards, 5 µg/kg and 30 µg/kg, respectively (Khalili et al., 2023). The levels of B[a]P and B[k]F in a variety of fish species ranged from 0.56 to 1.46 µg/kg when the fish was prepared by boiling, grilling, and frying in the Western Cape, South Africa (Olatunji et al., 2015). The levels of ∑2PAH were substantially lower in the grilled and boiled fish than in the fried fish ($p<0.05$) (Olatunji et al., 2015). This study agrees with the other two (Khalili et al., 2023; Olatunji et al., 2015) that the fish and meat products were safe for consumers because they all had less than the 5 µg/kg limit for B[a]P that is recommended for food.

Food prepared using heat treatment techniques, particularly grilling, char boiling, and braaing, has been correlated with high PAHs (Olatunji et al., 2015). PAHs infiltrate smoked items, where they are shielded from oxygen and light (Karl and Leinemann, 1996). Eventually, the concentration of these chemicals stabilizes at a consistent level (Karl and Leinemann, 1996). The levels of PAH in smoked fish products rise sharply during the smoking process and then gradually decline over time as a result of light degradation and interaction (Alcicek, 2011). However, low molecular weight PAHs are associated with this degradation. High-molecular-weight PAHs (HMW-PAHs), like pyrene, benzo[a]pyrene, and benzo[b]fluoranthene, are hard to dissolve and bioavailable. This means they don't break down easily, like when microbes attack them, and they stay in the environment for a long time. They also build up in animals (Raquel et al., 2013).

The incidence of PAHs in fish, meat, and poultry products has been found to vary greatly across different studies due to changes in processing, meat composition, and evaluation procedures. In addition to the smoking process (Drabova et al., 2013), other factors that impact contamination levels include the usage of vegetable oil (Stołyhwo and Sikorski, 2005). The strong lipophilic nature of PAHs makes them susceptible to movement and accumulation in edible oils and fatty meals (Wu et al., 2020). concentration in oils (0.095 µg/kg to 0.56 µg/kg) was previously reported to be seven to eleven times greater than that of smoked sprats (0.013 and 0.086 µg/kg) (Ciecierska and Obiedziński, 2007).

5. CONCLUSIONS

There were no statistically significant differences in the amounts of B[a]P and PAH4, plus probably- and potentially carcinogenic PAHs, among the different meat groups ($P > 0.05$). One beef sample had B[a]P levels over the 2 µg/kg MPL. Though one beef sample and three fish samples had unacceptable levels of B[a]P and B[k]F, respectively, 96.67% of the examined samples were safe according to safety requirements. To ascertain the potential health concerns connected to the intake of meat products by the Egyptian population, more extensive surveys that include the assessment of the margin of exposure and incremental lifetime cancer risk from various meat types should be conducted. Also, control and/or prevention trials are important to mitigate PAHs in meat.

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