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IMPACT OF POULTRY GROWTH STAGES ON TOXIC COMPOUNDS FORMATION DURING CHARCOAL GRILLING.

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ABSTRACT

Poultry is a widely consumed source of protein, with commercial production supplying chickens of varying weights to meet market demands. However, high-temperature cooking methods, such as charcoal grilling, have been associated with the formation of toxic Compounds, including polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). This study aimed to evaluate the impact of grilling on toxic Compounds formation in poultry of different weight categories. Chicken breast samples were categorized into three groups (A1, A2, A2+ as un-grilled and B1, B2, B2+ as grilled) and analyzed using High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS).

Results showed a significant increase in chromatographic peak areas after grilling, indicating the formation of potential toxic Compounds. The highest percentage increase (554%) was observed in the lowest weight category (A1 to B1), whereas the lowest increase (66%) was seen in A2 to B2. This suggests that the chemical composition and growth stage of poultry influence the extent of toxic Compounds formation during grilling. GC-MS analysis further identified specific compounds related to these changes.

The findings highlight the potential health risks associated with grilling poultry, emphasizing the need for controlled cooking methods to minimize exposure to harmful toxic Compounds. Future research should focus on alternative cooking techniques and risk mitigation strategies to enhance food safety.

KEYWORDS: Poultry, Toxic Compounds, charcoal grilling, HPLC, GC-MS, polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HCAs).

INTRODUCTION:

Poultry is one of the most widely consumed sources of animal protein, providing a fast-growing and commercially viable option for meeting global dietary needs. The poultry industry supplies chickens of varying weights to cater to market demands, with growth rates influenced by factors such as genetics, nutrition, and farming conditions. While chicken is considered a healthy protein source, the method of cooking can significantly impact its nutritional quality and safety (1-5).

Cooking methods such as grilling, frying, and roasting can lead to the formation of potentially harmful compounds, including toxic compounds. Among these, polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) are known to develop when meat is exposed to high temperatures or direct flames. Charcoal grilling, a popular cooking technique, has been particularly associated with an increased risk of toxic compounds formation due to the direct exposure of meat to smoke and heat (6-9).

A variety of feed and medicines used for meat development of poultry chickens so different weighted chicken seen in market. During growing periods (25 to 35 day of age), utility of essential amino acids leucine and valine was maximum as compared to starter periods (10 to 20 day of age). Breast weight gain was highest 24.4g/day at 47 day of growth than it declined after maturity. Charcoal grilling produced maximum PAHs as toxic compounds. Various organs showed different PAH's concentration but least was found in chicken chest (10-14).

Previous studies have indicated that factors such as cooking temperature, duration, and meat composition play a critical role in toxic compounds formation. However, limited research has explored the impact of poultry weight and growth stages on the development of these harmful compounds during grilling. This study aims to investigate the relationship between chicken weight and the formation of toxic compounds when subjected to charcoal grilling. By analyzing un-grilled and grilled chicken breast samples of different weights using HPLC and GCMS, we seek to determine whether variations in weight influence toxic compounds formation (15-19).

The findings of this study will contribute to a better understanding of how poultry growth stages affect food safety, helping consumers and the food industry make informed choices regarding poultry consumption and cooking methods.

MATERIAL AND METHOD:

SAMPLE COLLECTION AND PREPARATION:

Six poultry chicken samples of different weights were collected for the study. The samples were categorized into three groups: A1 & B1, A2 & B2, and A2+ & B2+. The gross weight (with feathers) and net weight (without feathers) of each sample are provided in Table 1.

Table 1: Gross and Net Weights of Poultry Samples

Samples	Gross weight (with feather)	Net weight (without feather)
A1 & B1	1270 grams	932 grams
A2 & B2	1822 grams	1352 grams
A2+ & B2+	2255 grams	1698 grams

Samples A1, A2, and A2+ were kept un-grilled, while B1, B2, and B2+ were subjected to charcoal grilling. For each sample, 20 g of chicken breast tissue was collected and prepared for further analysis.

GRILLING PROCESS:

The grilling of the B-series samples (B1, B2, B2+) was performed using a charcoal grill to simulate real-world cooking conditions. The grilling process was standardized to ensure uniform cooking. The samples were exposed to direct charcoal heat at a temperature range of 250–300°C for a duration of 10 minutes, ensuring even cooking on both sides. After grilling, the samples were allowed to cool at room temperature before further processing.

TOXIC COMPOUNDS EXTRACTION PROCEDURE:

A 20 g homogenized portion of each grilled and un-grilled sample was taken in a 500 mL round-bottom flask containing 50 mL of 2M potassium hydroxide (KOH) solution in a methanol: water (9:1) mixture. The mixture was subjected to saponification under reflux for two hours in a silvery water bath maintained at 70°C.

After cooling, 50 mL of n-hexane was added to each flask, and the mixture was stirred for 15 minutes. Subsequently, 50 mL of distilled water was introduced, and the solution was allowed to stand overnight to facilitate phase separation. The n-hexane layer was carefully collected using a separating funnel. The aqueous layer was then extracted twice with small portions of n-hexane to ensure maximum recovery of organic compounds.

To remove residual water, 2 g of anhydrous sodium sulfate was added to the collected n-hexane extracts. The extracts were then filtered and concentrated to 2 mL at 35°C using a rotary evaporator (Chung, 2011; B., 2011). The final concentrated extract was stored in a sealed vial for subsequent analysis.

CHEMICAL ANALYSIS:

The concentrated extracts from both grilled and un-grilled samples were subjected to HPLC & GCMS for toxic compounds detection. HPLC analysis was performed using a C18 reverse-phase column with a mobile phase composed of acetonitrile and water (80:20). The flow rate was maintained at 1.0 mL/min, and the detection was carried out using a UV detector set at 254 nm.

GC-MS analysis was performed to identify specific compounds formed due to grilling. The GC-MS system was equipped with an electron ionization source and separation was achieved using a DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The temperature program was set as follows: initial temperature of 60°C, ramped up to 280°C at a rate of 10°C/min, and held for 10 minutes.

STATISTICAL ANALYSIS:

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was carried out using ANOVA to determine significant differences between grilled and un-grilled samples. A p-value of <0.05 was considered statistically significant.

This methodology ensured proper extraction, isolation, and analysis of toxic compounds in different poultry weight categories. The results from HPLC and GC-MS provided insight into the impact of growth stage and grilling on toxic compounds formation.

RESULTS AND DISCUSSION:

PEAK AREA ANALYSIS OF UN-GRILLED AND GRILLED SAMPLES

PEAK AREA OF UN-GRILLED A1 AND GRILLED B1:

The percentage increase in peak area from un-grilled A1 to grilled B1 ranged from 96.93% to 3756.12%, with an average increase of 554.74%. This significant rise indicates a substantial formation of toxic compounds due to grilling. The highest percentage increase (3756.12%) was observed, demonstrating the impact of grilling on chemical composition.

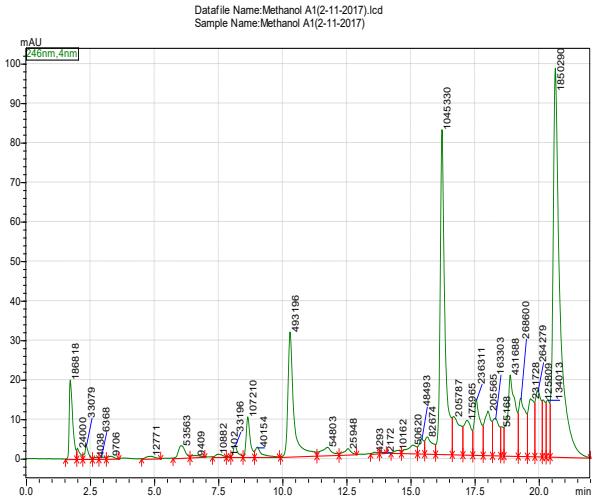

Figure A1: From HPLC equipment

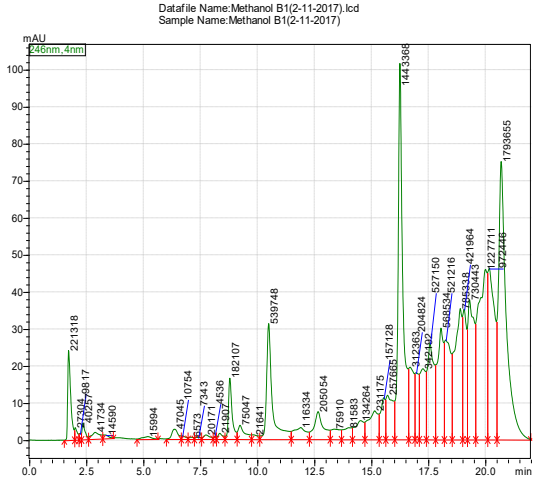

Figure B1: From HPLC equipment

Ungrilled	Grilled	%age inc
186818	221318	118.4672
24000	27304	113.7667
33089	40257	121.6628
6368	41734	655.3706
9706	14590	150.3194
107210	182107	169.8601
40154	75047	186.8979
493196	539748	109.4388
25948	205054	790.2497
4293	75910	1768.227
2172	81583	3756.123
10162	134264	1321.236
50620	231175	456.6871
48493	157128	324.022
82674	257665	311.6639
1045330	1443368	138.0777
205787	312363	151.7895
175965	342192	194.4659
236311	527150	223.0747
431688	785338	181.9226
268600	730443	271.9445
125809	1227711	975.8531
134013	972446	725.6356
1850290	1793655	96.93913
Average %age inc		**554.7373**

Table 2: Average peak areas of Un-Grilled A1 and B1 Grilled Samples

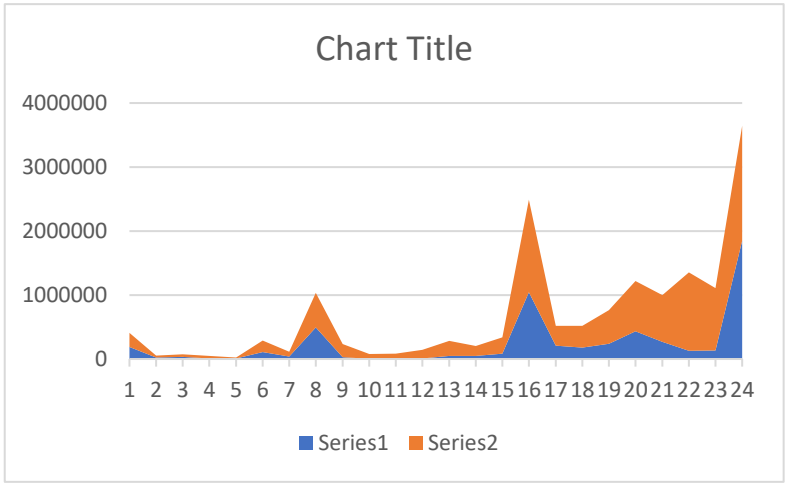

Chart 1: Average peak areas of Un-Grilled A1 and B1 Grilled Samples

Average percentage increase in peak area shows the increase in the expected toxic compounds in Un-Grilled A1 and B1 Grilled Samples (Table 2 & chart 1).

PEAK AREA OF UN-GRILLED A2 AND GRILLED B2:

The peak area analysis of A2 and B2 showed a lower overall increase compared to A1-B1, with values ranging from 27.79% to 134.23%, averaging 65.88%. The moderate increase suggests that differences in initial composition and weight influenced the toxic compounds formation during grilling.

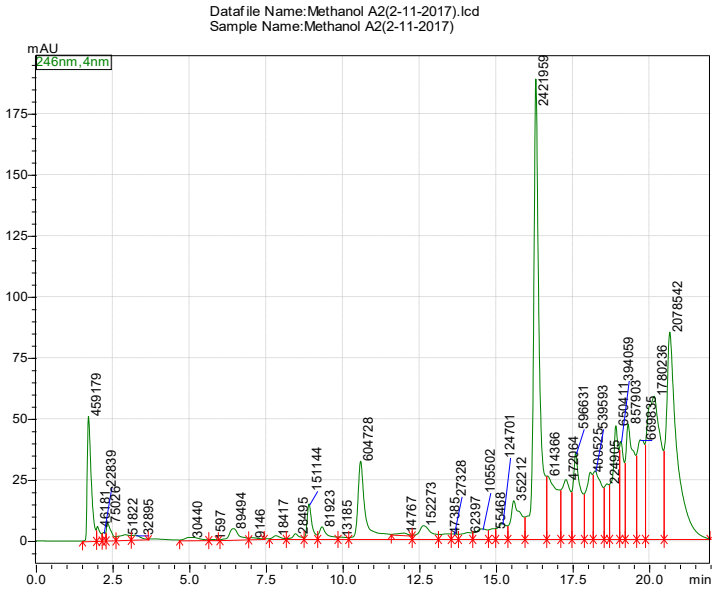

Figure A2: From HPLC equipment

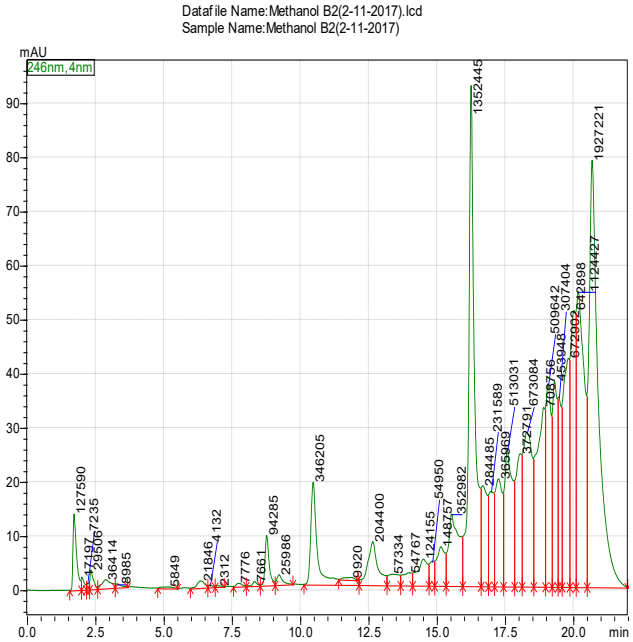

Figure B2: From HPLC equipment

Ungrilled	Grilled	%age inc
459179	127590	27.78655
75026	29506	39.3277
51822	36414	70.26745
89494	31846	35.58451
151144	94285	62.38091
81923	25986	31.72003
604728	346205	57.24971
152273	204400	134.2326
352212	352982	100.2186
2421959	1352445	55.84095
596631	513031	85.98799
1780236	1124427	63.16168
2078542	1927221	92.71985
Axerage %age inc		**65.88296**

Table 3: Average peak areas of Un-Grilled A2 and B2 Grilled Samples

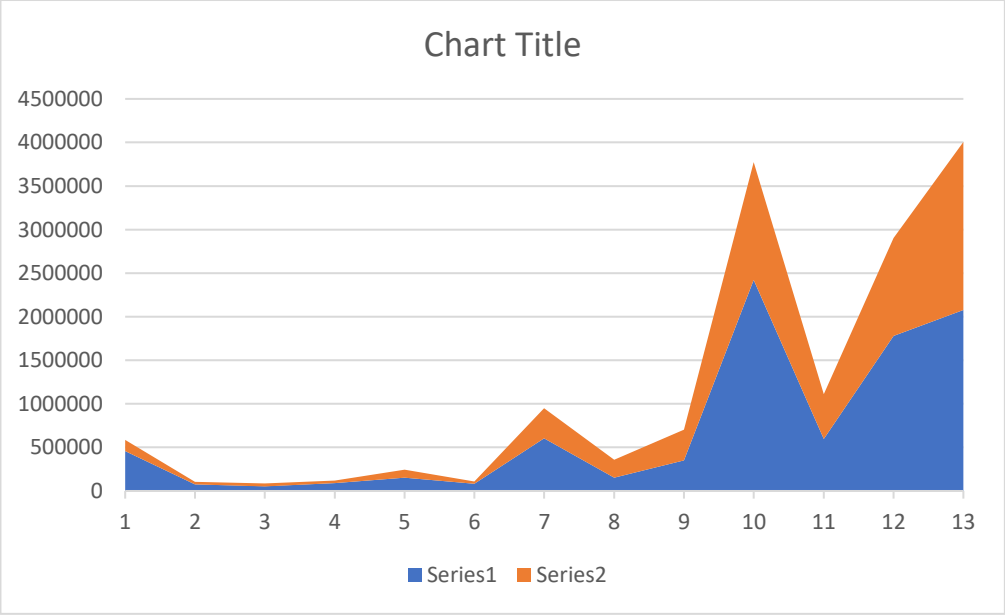

Chart 2: Average peak areas of Un-Grilled A2 and B2 Grilled Samples

Average percentage increase in peak area shows the increase in the expected toxic compounds in Un-Grilled A2 and B2 Grilled Samples (Table 3 & chart 2).

PEAK AREA OF UN-GRILLED A2+ AND GRILLED B2+

For A2+ and B2+, the percentage increase in peak area varied from 39.78% to 179.40%, with an average increase of 99.96%. While the overall increase was significant, it was lower than that observed in the A1-B1 comparison, indicating a potential relationship between growth stage and toxic compounds formation.

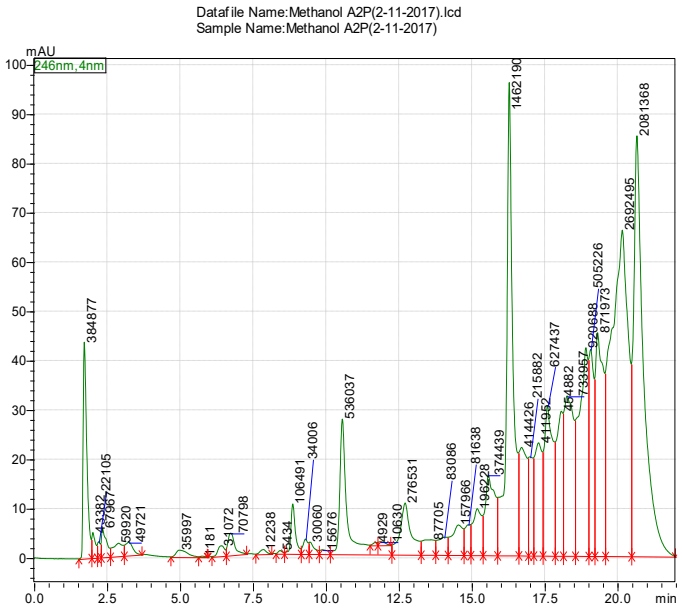

Figure A2+: From HPLC equipment

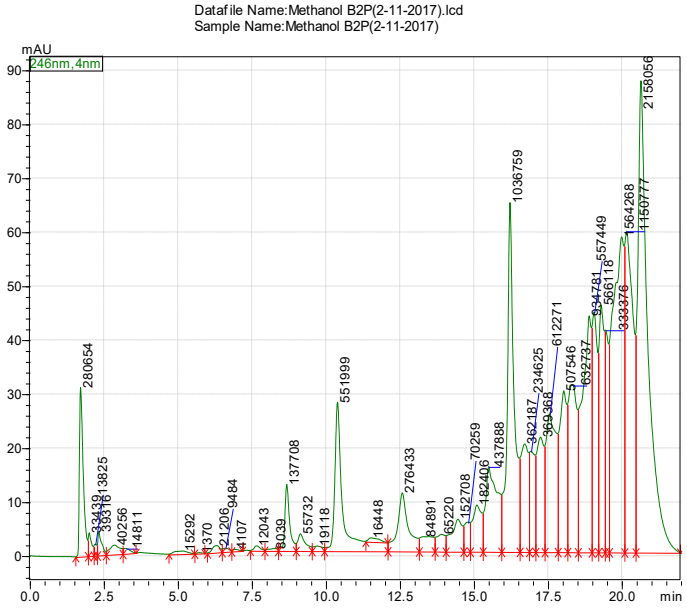

Figure B2+: From HPLC equipment

Ungrilled	Grilled	%age inc
384877	280654	72.92044
67967	39316	57.84572
59920	40256	67.18291
106491	137708	129.3142
34006	56732	166.8294
536037	551999	102.9778
276531	276433	99.96456
374439	437888	116.9451
1462190	1036759	70.90453
414426	362187	87.39485
215882	234625	108.6821
627437	612771	97.66255
454882	507546	111.5775
733957	632737	86.209
871973	1564288	179.3964
2892495	1150777	39.78493
2081368	2158056	103.6845
Axerage %age inc		**99.95744**

Table 4: Average peak areas of Un-Grilled A2+ and B2+ Grilled Samples

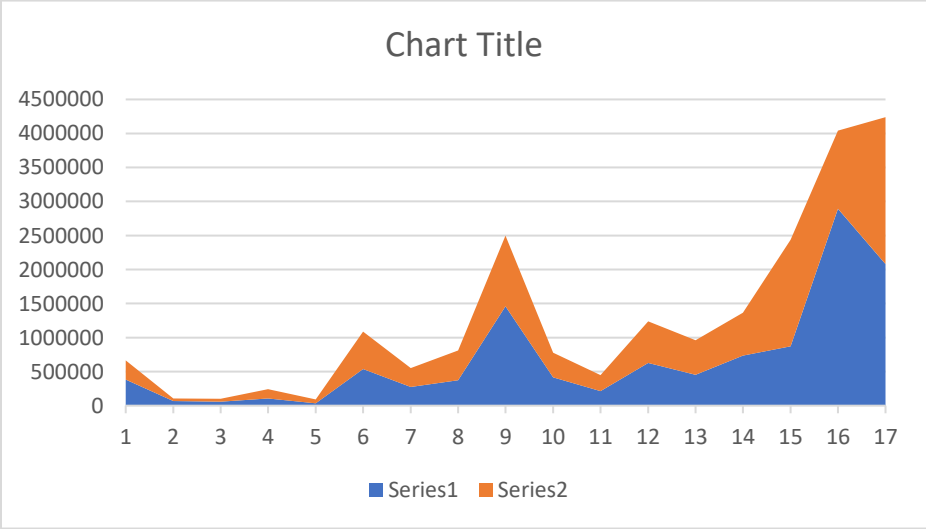

Chart 3: Average peak areas of Un-Grilled A2+ and B2+ Grilled Samples

Average percentage increase in peak area shows the increase in the expected toxic compounds in Un-Grilled A2+ and B2+ Grilled Samples (Table 4 & chart 3).

DISCUSSION:

The data indicate that grilling significantly increases toxic compounds formation, with the most substantial rise occurring in the lowest weight category (A1-B1). This suggests that younger or smaller chickens may be more susceptible to toxic compounds development upon grilling. The variations in peak area increase across different weight categories emphasize the influence of growth stages on chemical transformations during cooking.

These findings align with previous studies highlighting the role of high-temperature cooking in polycyclic aromatic hydrocarbon (PAH) and heterocyclic amine (HCA) formation. The results provide critical insights into the potential health risks associated with grilling poultry and emphasize the need for controlled cooking methods to minimize toxic compounds formation.

CONCLUSION:

The present study evaluated the impact of grilling on the formation of toxic compounds in poultry samples using High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). The results demonstrated a significant increase in peak area for various chemical compounds after grilling, suggesting the formation of potential toxic compounds. The extent of this increase varied among different weight categories, with the highest percentage increase observed in the smallest weight group (A1-B1). This indicates that the composition and growth stage of poultry may influence the extent of toxic compounds formation during grilling.

The findings of this study align with previous research highlighting the dangers associated with high-temperature cooking methods, particularly in the formation of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). While grilling is a widely preferred cooking method for enhancing flavor and texture, it is crucial to adopt safer cooking practices, such as marination, lower grilling temperatures, and indirect cooking methods, to minimize toxic compounds formation risks.

Future research should focus on identifying specific compounds formed during grilling and their potential health risks. Additionally, exploring alternative cooking techniques and their impact on toxic compounds formation could provide valuable insights into safer food preparation methods. These findings contribute to the growing body of evidence supporting the need for improved awareness and regulation of high-temperature cooking practices to safeguard public health.

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DISCLOSURE STATEMENT:

No potential conflict of interest.

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