Egyptian Journal of Veterinary Sciences

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Thermal Effect on Ractopamine Residues in Beef and Turkey Meat Using HPLC Assay

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Abstract

RACTOPAMINE (RAC) is commonly used in the US and other countries to enhance animal growth and lean body weight, but concerns have been raised about residues in edible tissues and potential adverse effects. Since most food is cooked before consumption, it is important to determine RAC's heat stability in these tissues. Therefore, the main goal of this research was to evaluate the heat stability of RAC in 100 samples of beef and turkey meat using high-pressure liquid chromatography (HPLC) with a UV detector. The results showed that RAC was not detected in any of the turkey muscle samples, while it was found in 22% of the beef muscle samples at levels ranging from 0.5 to 15 ppb, with an average of 6.4. Furthermore, 27.3% of the positive samples exceeded the permissible level. When examining the impact of various thermal methods (boiling, grilling, and frying) on RAC residues, it was observed that boiling, grilling, and frying reduced RAC levels by 17.3%, 42.1%, and 45.4%, respectively. The study also revealed that as the cooking temperature increased, the concentration of RAC decreased. The research concluded that RAC degradation varies depending on the degree of cooking temperature, and even after prolonged heating or high temperatures, only half of the remaining RAC in beef muscle was degraded. This suggests that traditional cooking methods may not completely eliminate RAC residues. Therefore, it is crucial to follow the recommended maximum residue limits for meat samples set by the Codex Alimentarius Commission, as meat is widely consumed and could pose health risks if contaminated with high levels of RAC.

Keywords: Ractopamine, β-agonist, Chromatography, Meat, Cooking.

Introduction

Ractopamine (RAC), a phenethanolamine β-adrenoreceptor agonist (β-agonist) is given to animals as a feed additive, it is related to the redistribution of nutrients by directing them from fat deposition to muscular tissue formation. The most significant risks associated with RAC residues are in the tissues. It can cause poisoning in humans leading to symptoms such as tachycardia, high blood pressure, headache, muscle spasms, tremors, apprehension, restlessness, and anxiety [1]. The U.S. Food and Drug Administration has authorized ractopamine for usage in turkeys since 2009, cattle since 2003, and pigs since 1999.

Maximum residue limits (MRLs) for ractopamine in turkey and beef muscle were set at 100 and 10 ppb, respectively, as recommended by FAO/WHO [2], Canada, the United States, and Japan; and adopted by CAC [3], where the maximum residue limits (MRL) acceptance in the United States, somewhere else have been either established or suggested in these

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(Received 13 March 2024, accepted 28 May 2024)
DOI: 10.21608/EJVS.2024.276665.1911

nations because of evaluations of human food safety. In a few countries, like China and the European Union, the use of β-agonists on food animals is forbidden. The residue marker of ractopamine is demarcated as the parent compound in all situations where MRLs have been established.

Various methods have been developed recently to detect and determine the presence of ractopamine residue. These methods include enzyme-linked immunosorbent assay (ELISA) [4, 5], gas chromatography-mass spectrometry [6] and high-pressure liquid chromatography [7, 8], and liquid chromatography coupled with mass spectroscopy (LC-MS) [9, 10].

HPLC determination after fluorescent or ultraviolet derivatization is preferred for its high precision [11]. Most labs use HPLC with a UV detector over GC-MS, LC-MS, LC-MS/MS, and UPLC-MS/MS due to its stability, adaptability, and low maintenance requirements. The viability and benefits of using 4-chloro-3,5-dinitrobenzotrifluoride (CDNT) as a derivatizing agent for amines have been shown in earlier studies. This reagent can provide adequate sensitivity when used with a basic UV detector, and the derivatized product is highly stable and shows good separation when using an HPLC system with a standard C18 column. Apart from the CDNT hydrolysis compound (CDNT-OH), the reaction contained no more byproducts or numerous derivatives., and the separation was not hampered by excess reagent or its hydrolysis product [12, 13] Furthermore, CDNT can be purchased commercially for less money than certain other biochemical labelling reagents.

This research aimed to measure the amount of ractopamine residues in turkey and beef meat using a verified chromatographic method while investigating the thermal effect through variable heat treatments.

Material and Methods

Meat specimens

Total of 100 beef and turkey meat samples (50, each) from different supermarkets.

Each sample was 500 grams and was taken to the laboratory for analysis. The samples were minced and weighted (5g) in 50 mL polypropylene tubes and maintained at -20^{o}C till HPLC analysis.

Blank beef and turkey meat were obtained from agriculture faculty farms, at Cairo University for standard curve preparation and method verification.

Thermal treatments

The cooked muscle was heated for approximately sixty minutes in a water bath at 100°C [14].

An electric grill that had been preheated to 150°C on the grill surface was used for grilling. Every two minutes, the grilled muscles were turned over after cooking for five minutes on each side [14].

On a home ceramic hob set to high heat, the muscle was fried in a quantity of sunflower oil for 4–6 minutes on each side, varying on the size of the sample [15].

HPLC instrument

HPLC quantification was done using quaternary pumps and multi wave detector; (AGILENT, Japan) set at 284 nm. Reversed phase (RP) technology was utilized for separation, using a Kromasil C18 ODS column with dimensions of 5 µm, 250 mm × 4.6 mm id, from the Netherlands at ambient temperature. The Chemstation software was used for method control and data analysis. The Oasis MCX 500 mg columns (J.T. Baker, Phillipsburg, USA) were also employed.

Chemicals

Ractopamine hydrochloride VETRANAL® standard (RAC- HCL) was purchased from Sigma-Aldrich (Milwaukee, WI). 4-chloro-3,5-dinitrobenzotrifluoride (CDNT) was provided by Alfa Aesar (Ward Hill, MA, USA). Trifluoroethanoic acid (TFA) and Sodium deuteroxide (HNaO) were obtained from Sinopharm Chemical Reagent Beijing., J.T. Baker (Phillipsburg, NJ, USA). HPLC grade methanol (MeOH), and deionized water from a Milli-Q system (Millipore, Billerica, MA, USA) were used. Other chemicals and solvents were of analytical grade and obtained from commercial sources. The 9.0 mM/L CDNT solution was formulated in methyl hydroxide.

The mobile phase (MPH) was degassed before injection into the HPLC system in a gradient mode as shown in Table 1. The run time is 15 minutes, and the post time is 5 minutes.

Standard preparation

The stock solution of 1000 ppm of RAC- HCL was kept in the ultrapure deionized water in a refrigerator at 4°C in the dark when not in use. The working standards were prepared in blank beef and turkey muscle (5-500 ppb), then prepared, purified, and derivatized as mentioned below.

Extraction

The extraction of ractopamine residue from various samples follows a three-step outlined in reference [16]: sample preparation, purification (SPE), and derivatization.

Sample preparation

Before beginning, the frozen samples were allowed to thaw at room temperature for approximately thirty minutes. Add 10 mL of 100.0 mM perchloric acid (PA) and mix for 45 min, then incubate in a water bath at 80°C for 20 minutes, followed by centrifugation for 5 minutes at 4500 rpm

using a cooling centrifuge. Afterward, the upper layer was filtered into a 50 mL P.P. tube. The residue was re-extracted with an additional 10 mL of 100.0 mM PA. The pH of the combined supernatants was adjusted to ten using a 5% HNaO solution. Then, 15 mL of Dimethyl carbinol– Acetoxyethane (1/9, v/v) was added, stirred vigorously, and centrifuged at 1000 rpm for 20 min. The supernatant was transferred to a 100 mL pear-shaped flask for re-extraction of residue with a solution of 10 mL Dimethyl carbinol– Acetoxyethane (1/9, v/v). A nitrogen stream was used to evaporate the combined organic extracts at 50°C until completely dry. The residue was dissolved in 5 mL of 0.02 M ammonium salt solution, centrifuged for 5 minutes at 5000 rpm, and purified using solid-phase extraction (SPE).

Sample Purification (SPE)

The cartridges were activated using 4 mL of MeOH and 5 mL of water. The extract was then slowly passed through the cartridge. The cartridge was washed with 2 mL of both water and MeOH and the column was dried for 3 minutes under pressure. The dried residue was eluted with 3 × 2.0 mL of 25% ammoniacal liquor in 5% MeOH. The eluent was dried using nitrogen flow. 100 µL of dibasic sodium phosphate, anhydrous (0.01 M) was used to dissolve the residue followed by derivatization.

Sample Derivatization

The CDNT and ractopamine reaction diagram is displayed in Fig. (1). A 1.0 mL vial was filled with 0.1 mL of the extract and 0.280 mL of MeOH in a specific order. The pH of the mixture was adjusted to 9.0 using a 0.5% HNaO solution. The vial was then vortexed for one minute. The CDNT solution (about 100 µL) was added and stirred again. The vial was placed in a thermostatic 70°C water bath for 10 minutes. Ten microliters of two-molar HCl were added to end the reaction. After cooling, 20 µL of the derivatized extract was injected into the HPLC device after being filtered through 0.45 µm nylon acrodiscs.

Method qualification

The calibration plot was set at 7 levels at 5, 10, 20, 50, 100, 200, and 500 ppb in different matrices (at a range of 5- 500 ppb) in a triplicate manner. The slope (a), intercept(b), and correlation (R) were determined. Both detection (LOD) and quantification limits (LOQ) were calculated according to, intercept standard deviation, (S), and slope (a) [LOD= 3.3×S/a; LOQ=10×S/a]. Also, recovery was detected after fortification of the blank matrices with ractopamine standard at 1/2 X, 1X, and 1.5X of MRL) as recommended [17].

Statistical analysis

The descriptive statistics and ANOVA test between the means was assessed using SPSS (20-SPSS, Chicago, USA). Statistics were significant at *P-value* ≤0.05 according to [18].

Results

Results of method qualification:

As indicated in Table (2), peak area versus concentration in samples of blank turkey and beef meat were plotted to create the calibration plots for RAC. The suggested method's linearity was examined in the 5–200 ppb range, with a 0.9999 of correlation coefficient (R^2). The linear regression equation was y = 20.01X – 2.5 for RAC in beef meat, whereas for RAC in turkey meat, it was y = 20.98X + 8.1 (Fig. 2).

The equilibrated chromatograms of RAC in blank samples of beef and turkey meat showed a distinct retention time of 6.213 min. There were no impurities or excipient interferences observed among the various extracted blank and spiked meat samples (Fig. 3).

Various standard concentrations are created by introducing known amounts of RAC onto blank muscle samples. These samples are then compared to standard solutions with identical concentrations. The accuracy of the analysis is determined by calculating the percentage recovery from the experimental data. The outcomes are presented in Table (3) indicating a high level of recovery. The detection and quantification limits were tabulated (Table 4).

Results of RAC residue in collected samples

It was not detected in all examined turkey breast muscle and detected in 22% of analyzed beef muscle (11 positive samples from 50) to found in the range of 0.5 to 15 ppb at an average of 6.4 (Table 5). It was found that 27.3% of the positive samples exceeded the permitted level set by [3].

It was found that the mean of RAC declined to be 5.3± 3.9 after boiling, 3.8± 3.1 after grilling, and 3.8± 3.2 post frying as shown in Fig. (4) and Table (6) to be the reduction percentage of 17.3, 42.1, and 45.4 % after boiling, grilling, and frying, respectively (Fig. 5).

Discussion

In the current investigation, a verified high-pressure liquid chromatography (HPLC) assay with a UV detector was used to quantify ractopamine residues in beef and turkey meat. Recently, HPLC assays have gained recognition as reliable and cost-effective techniques for detecting ractopamine residues in both imported and exported meat. Our experimental procedure underwent rigorous verification, and the correlation coefficient obtained from the linearity analysis exceeded 0.9999. The recovery rates for ractopamine from beef muscle

ranged from 96.1% to 98.1%, while for turkey meat, the recovery rates ranged from 91.9% to 94.8%. These findings align with the acceptable criteria recommended by [17]. The limits of detection (LOD) for ractopamine residues in beef and turkey meat were determined to be 0.05 and 0.1 parts per billion (ppb), respectively. These values fall below the maximum residue limit (MRL), indicating that the developed method exhibits exceptional sensitivity and is well-suited for monitoring ractopamine residues in these matrices. To ensure accurate and straightforward analysis with high recovery rates, the sample components were purified using solid-phase extraction (SPE) cartridges.

No traces of ractopamine were found in the turkey breast meat samples examined during this investigation. These findings assure the public that there are no anabolic agent residues in turkey meat, which can be attributed to the vigilance of veterinarians and the effective management of farms in terms of drug residues and adherence to relevant regulations [19].

It was detected that 22% of analyzed beef muscle was found in a range of 0.5 to 15 ppb at an average of 6.4. It was found that 27.3% of the positive samples exceeded the permitted level set by Fang et al., [4]. The findings of our study surpassed the results obtained by Hassan et al., [20], wherein they reported that the average levels of ractopamine in beef samples were 3.43±0.43 µg/kg, with a range of 1.90 to 5.00 ppb. The disparity in our results could potentially be attributed to variances in the analytical methodology employed [21].

The findings demonstrated the influence of variable thermal treatments on the presence of ractopamine deposits in analyzed meat samples when subjected to boiling, grilling, and frying. The concentration of ractopamine residues following boiling for 60 minutes at 100°C was lowered to 5.3±3.9 ppb, with a reduction rate of 17.3%. This outcome aligns with the results informed by [22], who observed a 17.9% mean degradation rate in ractopamine residues within beef tissue (muscle) following boiling. Similarly, **[**20**]** noted a degradation rate of 19.24% for ractopamine after boiling. In contrast, [21] claimed that even after boiling in water, the residues of β-agonists stayed unchanged.

The levels of ractopamine residues in beef decreased after grilling and frying cooking methods, as indicated by the decline in percentages (42.1, 45.4%). This finding aligns with the research conducted by Sornprasit, [23], which demonstrated a 50% decrease in β-agonist residues when beef was roasted at 200°C for approximately 10 minutes. In contrast, [24] revealed a reduction of 99.88% in RAC residues when barbecuing for 15 minutes at 200°C. Based on these results, it is recommended that grilling be considered as the optimal cooking method for reducing ractopamine residues in beef, but not eradicate it completely.

Ractopamine has the ability to permeate deep tissues within vital organs like the liver, spleen, and kidneys, thereby heightening the likelihood of tumors [25]. Hence, it is crucial to recognize the potential for residues in animals that are slaughtered prior to the completion of the withdrawal time. Remarkably, RAC residues partially disappear at temperatures between 100 and 120^{o}C. Thus, inadequately cooked meat may result in the ingestion of these residues. Consequently, consistent consumption of such residues is likely to elevate the risk of cancer.

The U.S. Food and Drug Administration has asserted that humans consuming ractopamine residues at permissible doses have a short-range negative impact on health in response to public health concerns. Ractopamine hastens the development of endothelial dysfunction, atherosclerosis, disruption of cholesterol metabolism, tumor proliferation, and cardiovascular system disorders [26, 27, 28]. Food contaminated with ractopamine poses a risk of vomiting, dizziness, muscle pain, kidney damage, heart disease, and even death in humans [29, 30]. Heart palpitations, nausea, anxiety, higher blood pressure, restlessness, vasodilation, and muscle tremors were also recorded [31, 32].

Conclusions

A study analyzed ractopamine levels in turkey and beef meat samples using a verified procedure. Recovery rates for ractopamine in beef muscle ranged from 96.1% to 98.1%, while in turkey meat, it ranged from 91.9% to 94.8%. Accurate detection of ractopamine residues is feasible, demonstrating the importance of effective monitoring measures. In turkey muscle samples, ractopamine was not detected but was found in 22% of samples. Heat treatment, such as boiling, grilling, and frying, resulted in degradation percentages of 17.3%, 42.1%, and 45.4%, respectively. However, only half of the remaining ractopamine was degraded after two hours of continuous heating at elevated temperatures. Adherence to the Codex Alimentarius Commission's recommended maximum residue limits in meat samples is crucial.

Recommendations

To reduce the residues of ractopamine and ensure they remain at safe levels for human consumption, the following points should be considered:

-Animals should not be slaughtered before the completion of the withdrawal period for ractopamine.

-Implement intensive national inspections for ractopamine residues.

-Ensure the meat is adequately cooked.

Acknowledgment

All great thanks to the Department of Chemistry, Toxicology, and Feed Deficiency at the Animal Health Research Institute for their excellent support and completion of this study.

Conflicts of interest

According to the authors, there isn't a conflict of interest.

Funding statement

There's no funding source.

TABLE 1. Gradient mobile phase for ractopamine separation.

Time	MPH. A [MeOH]	MPH. B [0.1% TFA]	Flow rate
0	80	20	1.2 ml/min.
6	20	80	
9	20	80	
14	80	20	

TABLE 2. The levels of ractopamine (ppb)and their respective area under peak (AUP) values were determined through HPLC analysis.

RT	Signal	Compound	Amt. [ppb]	AUP/ Beef muscle	AUP/ Turkey muscle
6.213	284 nm	RAC- CDNT	5	103.7	105.7
			10	196.9	214.2
			20	397.8	427
			50	1008.2	1060
			100	1968.2	2140
			200	4013	4186

TABLE 3. Recovery results of ractopamine from fortified blank beef and turkey samples.

Muscle	Conc. (ppb)	Mean ± SD
Beef	15	98.1± 0.8
	30	96.1± 0.4
	60	96.8± 0.3
Turkey	50	94.8± 0.5
	100	93.1± 0.4
	200	91.9± 0.7

TABLE 4. Limit of detection (LOD) and quantification (LOQ) of HPLC assay of ractopamine.

Matrix	LOD (ppb)	LOQ (ppb)
Beef muscle	0.05	0.1
Turkey muscle	0.1	0.2

TABLE 5. Ractopamine residues in beef muscle samples

Sample no.	RAC conc. (ppb)
1	0.5
2	2
3	3.1
4	3.1
5	12*
6	9
7	10.2*
8	15*
9	1.5
10	5.3
11	8.2
Mean± SD	6.4± 4.8

*Exceed MRL according to [**3**].

TABLE 6. The impact of thermal treatments on the levels of ractopamine (ppb) in meat samples.

Sample no.	Raw	Boiling	Grilling	Frying
1	0.5	0.42	0.27	0.2
2	2	1.4	0.9	0.8
3	3.1	2.7	2.1	2
4	3.1	2.6	1.8	1.7
5	12	10	7.2	7.2
6	9	7.8	5.2	5.2
7	10.2	8.2	6.2	6.3
8	15	12.1	10.3	10.2
9	1.5	1.3	0.8	0.7
10	5.3	4.3	3.2	3
11	8.2	7	4.2	4.1
Mean± SD	6.35± 4.8	5.26± 3.9	3.83± 3.1	3.76± 3.2

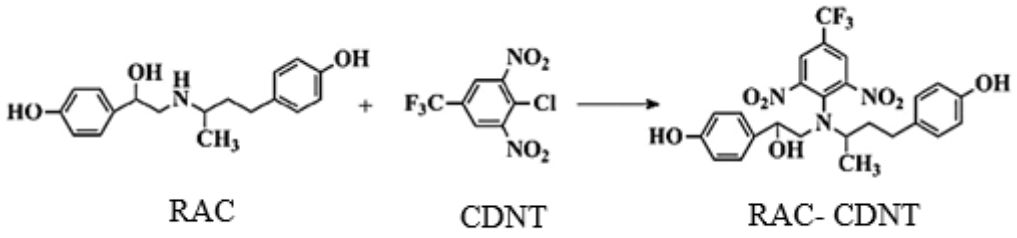

Fig. 1. Derivatization reaction diagram of ractopamine.

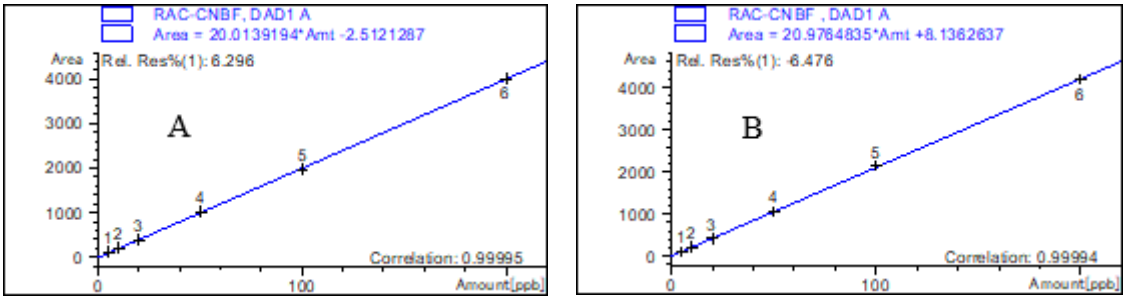

Fig. 2. Calibration curves of ractopamine in beef (A) and turkey (B) meat

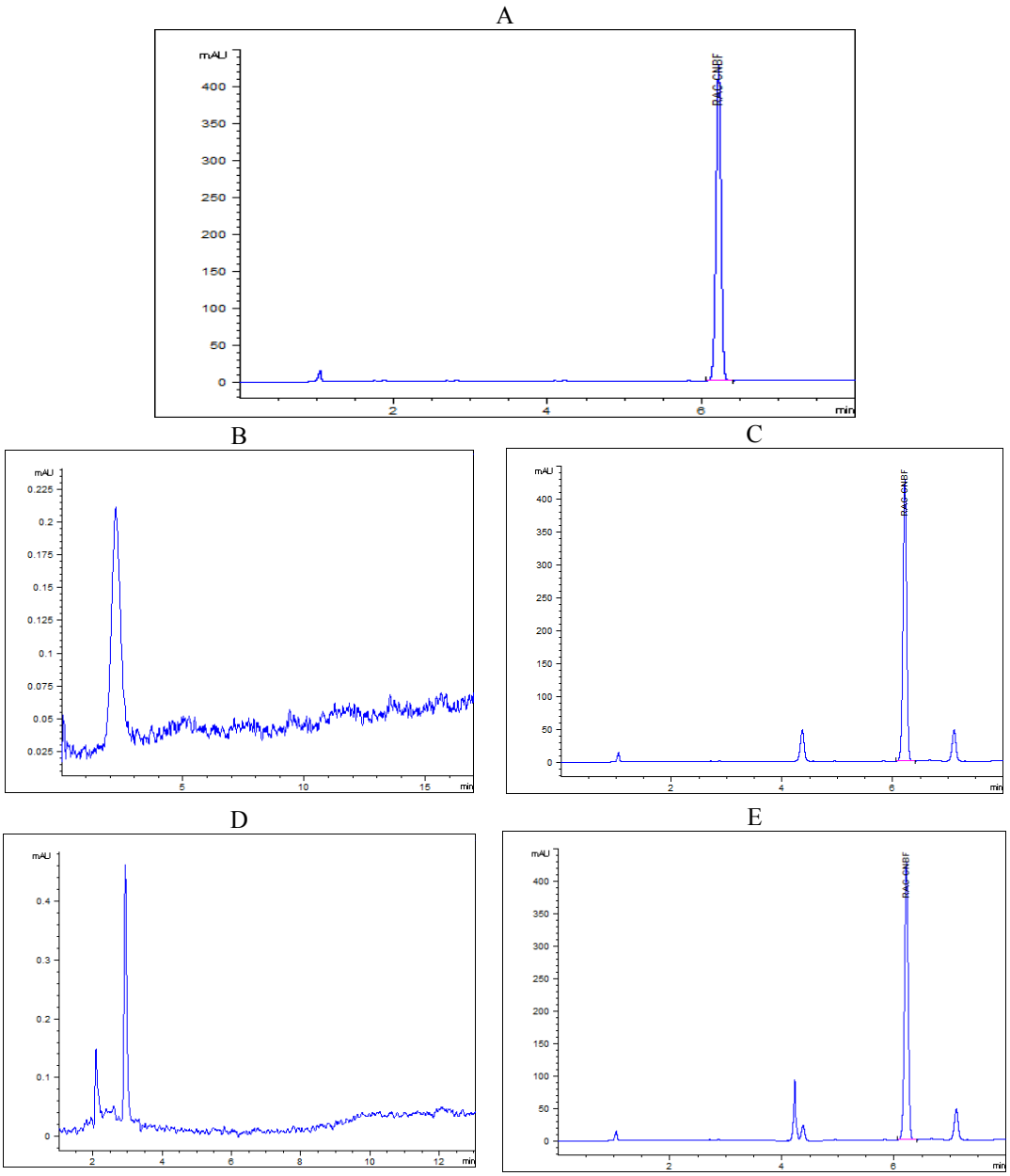

Fig. 3. Chromatograms of RAC at a level of 100 ppb in water (A), beef (C), and turkey (E) blank meat; chromatograms of blank beef (B) and blank turkey meat (D)

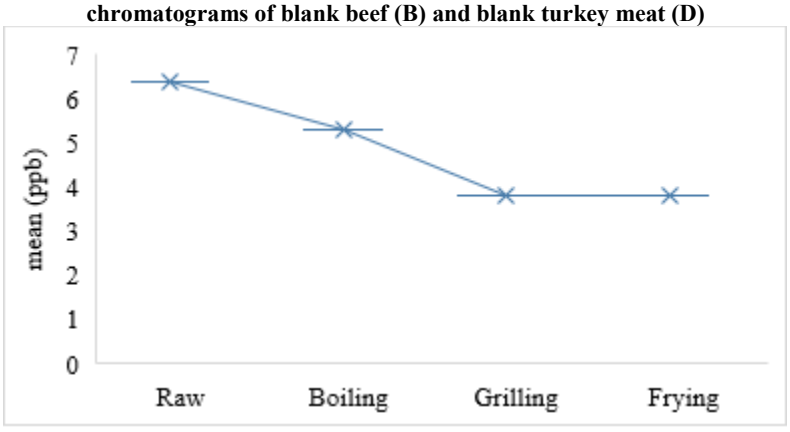

Fig. 4. Ractopamine concentrations after heat treatment.

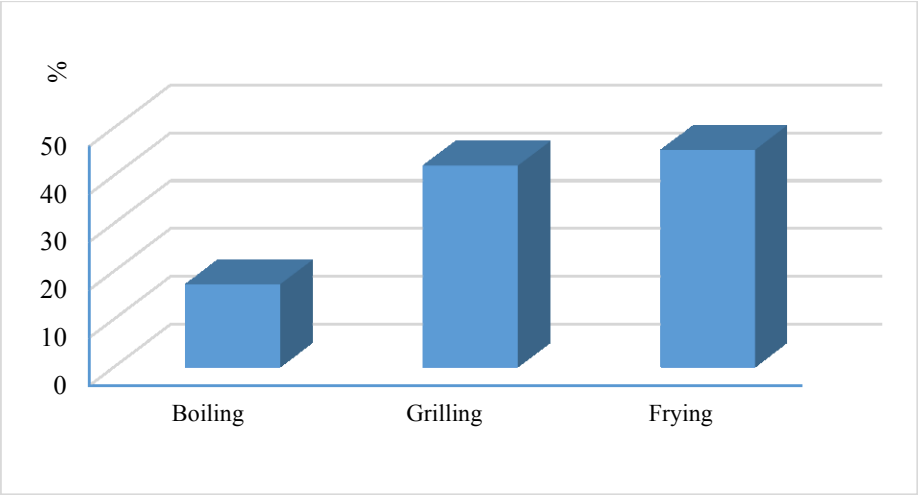

Fig. 5. Reduction percentage of ractopamine after cooking

References

1. European Food Safety Authority (EFSA). Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety evaluation of ractopamine/*The EFSA Journal*, **1041**, 1-52 (2009).
2. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission. Thirty-fifth Session FAO Headquarters, REP12/CAC Rome, Italy, 87-120 (2012).
3. Codex Alimentarius Commission (CAC) adopts maximum residue levels. UN food safety body sets limits on veterinary growth promoting drug 06-07-2012 (2012).
4. Fang, G., Lu, J., Pan, M., Li, W., Ren, L. and Wang, S. Substitution of antibody with molecularly imprinted film in enzyme-linked immunosorbent assay for determination of trace ractopamine in urine and pork samples. *Food Analytical Methods*, **4**, 590-597 (2011).
5. Peng, D., Zhao, L., Zhang, L., Pan, Y., Tao, Y., Wang, Y. and Yuan, Z. A novel indirect competitive enzyme-linked immunosorbent assay format for the simultaneous determination of ractopamine and phenylethanolamine, a residue in swine urine. *Food Analytical Methods*, **12**, 1077-1085 (2019).
6. Zhu, S., Mou, B., Zheng, L., Wen, L., Gan, N. and Zheng, L. Determination of β2-Agonist Residues in Meat Samples by Gas Chromatography-Mass Spectrometry with N-Doped Carbon Dots in Molecular Sieves. *Separations*, **10**(8), 429 (2023).
7. Peng, C., Zhang, S., Wu, C., Feng, Y., Zhao, D., Wang, X. and Bai, Z. In-tube solid phase microextraction and determination of ractopamine in pork muscle samples using amide group modified polysaccharide-silica hybrid monolith as sorbent prior to HPLC analysis. *Food Chemistry*, **355**, 129662 (2021).
8. Ar-sanork, K., Karuwan, C., Surapanich, N., Wilairat, P., Nacapricha, D. and Chaisuwan, P. Mixed mode monolithic sorbent in pipette tip for extraction of ractopamine and clenbuterol prior to analysis by HPLC-UV and UHPLC-Q ExactiveTM Plus Orbitrap MS. *Journal of Analytical Science and Technology*, **12**(1), 23 (2021).
9. Gao, H., Chen, M., Gao, H., Gao, S., Liu, X., Du, R. and Wan, L. Determination of ractopamine residue in animal derived foods using electro membrane extraction followed by liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, **1675**, 463179 (2022).
10. Shelver, W. L., McGarvey, A. M., Holthusen, J. E., Young, J. M., Byrd, C. J. and Smith, D. J. Comparison of immunoassay and LC-tandem mass spectrometry analyses of ractopamine in hog oral fluid. *Food Additives & Contaminants:* Part A, 1-13 (2024).
11. Cevasco, G., Piątek, A. M., Scapolla, C. and Thea, S. An improved method for simultaneous analysis of aminothiols in human plasma by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A*, **1217**(14), 2158-2162 (2010).
12. Tang, T., Shi, T., Qian, K., Li, P., Li, J. and Cao, Y. Determination of biogenic amines in beer with pre-column derivatization by high performance liquid chromatography. *Journal of Chromatography B*, **877**(5-6), 507-512 (2009).
13. He, S., Chen, Q., Sun, Y., Zhu, Y., Luo, L., Li, J., and Cao, Y. Determination of tobramycin in soil by HPLC with ultrasonic-assisted extraction and solid-phase extraction. *Journal of Chromatography B*, **879**(13-14), 901-907 (2011).
14. Choi, Y. S., Hwang, K. E., Jeong, T. J., Kim, Y. B., Jeon, K. H., Kim, E. M. and Kim, C. J. Comparative study on the effects of boiling, steaming, grilling, microwaving and superheated steaming on quality characteristics of marinated chicken steak. *Korean Journal for Food Science of Animal Resources*, **36**(1), 1-7 (2016).
15. Cooper, K. M., Whelan, M., Danaher, M. and Kennedy, D. G. Stability during cooking of anthelmintic veterinary drug residues in beef. *Food Additives and Contaminants*, **28**(2), 155-165 (2011).
16. Ding, G., Li, D., Qin, J., Zhu, J., Wang, B., Geng, Q. and Cao, Y. Development and validation of a high-

performance liquid chromatography method for determination of ractopamine residue in pork samples by solid phase extraction and pre-column derivatization. *Meat Science*, **106**, 55-60 (2015).

17. ICH Harmonized Tripartite Guideline. Validation of analytical procedures: text and methodology. *Q2 (R1),* **1**(20), 05 (2005).
18. Strunk, K. K. and Mwavita, M. Design and analysis in educational research: ANOVA designs in SPSS®. Routledge (2020).
19. Cayci, M., Celik, A. S., Oruc, H. H. and Sariyev, R. Screening of veterinary growth-promoting agent and antibacterial residues in beef cattle and broiler meats consumed in Bursa, Turkey. *Journal of Research in Veterinary Medicine*, **38**(1), 52-58 (2019).
20. Hassan, M. A., Reham, A.A., Marzouk, N. M. and Sarah, R.kh. Effect of different cooking methods on ractopamine residues in beef. *Benha Veterinary Medical Journal*, **31**(2), 210-212 (2016).
21. Rose, M. D., Rowley, L., Shearer, G. and Farrington, W. H. Effect of cooking on veterinary drug residues in food. 6. Lasalocid. *Journal of Agricultural and Food Chemistry*, **45**(3), 927-930 (1997).
22. Chang ShaoKuang, C. S., Hsieh MingKun, H. M., Lee WeiMing, L. W., Lan PeiYu, L. P., Zhang WenFa, Z. W. and Chou ChiChung, C. C. Evaluation of the heat stability of ractopamine in beef, pork, swine liver, swine kidney and swine lung. *Taiwan Veterinary Journa*l, **37**(4), 253-261 (2011).
23. Sornprasit, S. Effect of heating on salbutamol residue in raw pork tissue. *Warasan Krom Witthayasat Kan Phaet*. *Journal of Environmental Management,* **1**(1),1-11(2005).
24. Marzouk, N.M. Effect of Different Heat Treatment on Ractopamine Residue in Cattle`s Meat, Liver and Kidney. *J. of Appl. Sci*., **27**(12B), 297-307 (2012).
25. Grandin, T. On-farm conditions that compromise animal welfare that can be monitored at the slaughter plant. *Meat Science*, **132**, 52-58 (2017).
26. Zaitseva, N. V., Shur, P. Z., Atiskova, N. G., Kiryanov, D. A. and Kamaltdinov, M. R. Health risk assessment of exposure to ractopamine through consumption of meat products. *Int. J. Adv. Res. (Indore*), **2**(9), 538-545 (2014).
27. Chen, C. H., Guo, B. C., Hu, P. A., Lee, H. T., Hu, H. Y., Hsu, M. C. and Lee, T. S. Ractopamine at legal residue dosage accelerates atherosclerosis by inducing endothelial dysfunction and promoting macrophage foam cell formation. *Environmental Pollution*, **313**, 120080 (2022).
28. Fan, F. S. Consumption of meat containing ractopamine might enhance tumor growth through induction of asparagine synthetase. *European Journal of Cancer Prevention*, **31**(1), 82 (2022).
29. He, L., Su, Y., Zeng, Z., Liu, Y., and Huang, X. Determination of ractopamine and clenbuterol in feeds by gas chromatography–mass spectrometry. *Animal Feed Science and Technology*, **132**(3-4), 316-323 (2007).
30. Divari, S., Berio, E., Pregel, P., Sereno, A., Chiesa, L., Pavlovic, R. and Cannizzo, F. T. Effects and detection of nandrosol and ractopamine administration in veal calves. *Food Chemistry*, **221**, 706-713 (2017).
31. Shieh, J. S. and Yang, S. G. Statutory safety quarantine and its compensation of consumer's long-term intake of food additives. *Journal of Agriculture and Food Research*, **7**, 100264 (2022).
32. Aroeira, C. N., Feddern, V., Gressler, V., Contreras-Castillo, C. J. and Hopkins, D. L. A review on growth promoters still allowed in cattle and pig production. *Livestock Science*, **247**, 104464 (2021).

التأثير الحراري على بقايا الراكتوبامين في لحم البقر والديك الرومي باستخدام جهاز HPLC.

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الملخص

يستخدم الراكتوبامين (RAC) بشكل شائع في الولايات المتحدة وبلدان أخرى لتعزيز نمو الحيوانات ووزن الجسم النحيل، ولكن أثيرت مخاوف بشأن المخلفات في الأنسجة المستهلكة والآثار الضارة المحتملة. بما أن معظم المواد الغذائية تخضع للطهي، فمن الأهمية بمكان تحديد استقرار حرارة RAC في الأنسجة الصالحة للأكل.

ومن ثم، كانت أهداف البحث الأولية هي تقييم ثبات الحرارة RAC في 100 عينة من لحم البقر والديك الرومي من خلال استخدام التحليل اللوني السائل عالي الضغط (HPLC) المتصل بكاشف الأشعة فوق البنفسجية.

أشارت النتائج إلى أنه لم يتم اكتشاف RAC في جميع عضلات الديك الرومي التي تم فحصها، وتم اكتشاف 22٪ من عضلات لحم البقر التي تم تحليلها في نطاق يتراوح من 0.5 إلى 15 جزء في البليون بمتوسط 6.4. وتبين أن 27.3% من العينات الإيجابية تجاوزت المستوى المسموح به. وحول تأثير الطرق الحرارية المختلفة على بقايا الـ RAC (الغلي، والشوي، والقلي)، أشارت النتائج إلى أن نسبة التخفيض في الـ RAC بعد الغليان، والشوي، والقلي كانت 17.3%، 42.1%، و45.4% على التوالي. أشارت النتائج إلى أنه مع زيادة درجة التسخين، كان هناك انخفاض في تركيز RAC.

وجدت الدراسة أن تحلل RAC يختلف عبر المصفوفات، وحتى بعد ساعتين من التسخين المستمر أو درجات الحرارة المرتفعة، تم تحلل فقط نصف RAC المتبقي في عضلات اللحم البقري، مما يشير إلى أن طرق الطهي التقليدية لا يمكن أن تقلل تمامًا من بقايا RAC. وأخيراً، من الضروري الالتزام بالحدود القصوى الموصى بها لمخلفات عينات اللحوم التي اقترحتها هيئة الدستور الغذائي، لأنها عضو يتم استهلاكه على نطاق واسع في جميع أنحاء العالم وقد يسبب مشاكل صحية للإنسان عند استهلاكه بتركيزات عالية.

الكلمات الدالة: راكتوبامين، ناهض بيتا، HPLC، اللحوم، الطهى.