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CONTROL OF RACTOPAMINE RESIDUES IN IMPORTED FROZEN BEEF AND LIVER USING DIFFERENT COOKING METHODS

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ABSTRACT:

This study was performed to detect the ractopamine residue in 70 samples of imported frozen meat and liver, with 35 samples from each type. Samples were collected from different frozen meat stores in Assiut City and Assiut University. Results of RAC residues indicated that 94.3% of samples of imported frozen beef were accepted and 5.7% were unaccepted and above the MRL of RAC as set by the National Food Safety Authority (NFSA, 2020). Regarding frozen liver, 97 % of samples were accepted, and 3% were unaccepted according to the National Food Safety Authority (NFSA, 2020). In conclusion, the amount of β-agonist RAC residues detected in imported frozen meat and liver is safe.

Furthermore, different cooking methods were applied to samples of meat and liver with known concentrations of ractopamine (4 ppb) to detect the effect of heat treatment on RAC residues. Meat samples were subjected to cooking by boiling at 100 °C for 120 min, grilling at 200 ºC for 15 min, and Microwave at 150-180 °C for 8 min. Liver samples were cooked by grilling at 170–200 °C/6 min, frying at 230 °C for 16 min, and microwave at 205 °C for 8 min. The most effective cooking methods for reducing RAC residue were microwave, followed by boiling, frying, and then grilling.

Keywords: Ractopamine residues, frozen meat, frozen liver, cooking microwave.

INTRODUCTION

The frozen meat outlets sell imported frozen meat and liver in reasonable amounts and at competitive prices. Brazilian frozen beef and American frozen liver are the most popular imported meat and liver varieties in the Egyptian market. Also, Brazilian frozen liver and Indian frozen buffalo meat are available. Ractopamine, a β1/2-adrenergic receptor agonist, has been approved for use as a feed additive in finishing cattle, turkey, and swine in countries where meat exporting brings tremendous economic benefits to increase growth rate and carcass lean percentage (leanness-enhancing agent) (Huang *et al.*, 2016 and Fan, 2023).

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Historically, the Food and Drug Administration (FDA) determined that RAC was safe in 1999 and authorized its use in pig feed. In 2003, the FDA allowed its use in cattle feed, and in 2008, it was approved for use in turkey feed. No withdrawal time is required for this FDA approval (US-FDA, 2014). The Ministry of Agriculture, Livestock, and Food Supply (MAPA) of Brazil approved using RAC on feedlot cattle in 2011. Numerous studies on ractopamine's safety have been reviewed by the United States Food and Drug Administration (FDA). They have found that when cattle are fed ractopamine by the manufacturer's recommendations, the meat from those animals is safe for human consumption.

Ractopamine in muscle (meat) metabolizes and is eliminated quickly; therefore, the product can be fed till harvest (Lynch *et al.*, 2022). Furthermore, Fan (2023) found that the risk of type 2 diabetes can be reduced by long-term consumption of meat containing ractopamine.

In 2012, Codex Alimentarius set maximum residue limits (MRL) for ractopamine in pork and beef muscle at 10 parts per billion (ppb) and in beef liver at 40 ppb (CAC, 2017). In contrast, the US Food and Drug Administration (FDA) established MRLs for ractopamine in beef muscle and liver at 30 ppb and 90 ppb, respectively (US FDA, 2020). These values are significantly higher than those recommended by Codex. The National Food Safety Authority (NFSA) of Egypt published Decision No. 13/2020 on November 15, 2020. It established the Codex MRL guideline for muscle cuts (10 ppb). It set a tolerance level of 20 parts per billion (ppb) for the residual of ractopamine in the liver (USDA Foreign Agricultural Service, 2021).

The researchers were driven to guarantee the safety of the levels of RAC hormone in imported frozen meats by public knowledge of the potential health hazards associated with the residues of such substances. Therefore, the present study was conducted to determine the residues of RAC hormone in imported frozen beef and liver and investigate the effect of various popular cooking methods on reducing or eliminating RAC residue in meat and liver.

MATERIALS AND METHODS:

1. Collection of samples:

Seventy frozen meat and frozen liver samples (35 samples each) were collected randomly from different frozen meat stores in Assiut City and Assiut University dormitory in the period between October/2019 to December/2019. The samples of frozen meat included 28 samples of Brazilian meat, and 7 samples of Indian Meat, while the samples of frozen liver consisted of 24 samples of American liver, 8 samples of Brazilian liver, and 3 samples of Indian liver. Each sample was obtained in a separate plastic bag. The collected samples were stored in the freezer at -18 º C for further analysis.

Each sample was obtained in a separate plastic bag. The collected samples were stored in the freezer at -18º C for further analysis.

2. Detection of Ractopamine in Frozen Beef and Liver Samples:

2.1. Preparation of samples:

At the laboratory, the samples were thawed in the refrigerator at 4 ºC overnight. After thawing, each sample was aseptically and carefully freed from its plastic bag and cut into fine particles by a sterile scissor. The fat was removed, and the sample was homogenized to a fine mass using a stomacher or mixer.

Then the steps were followed as mentioned in the attachment for the ELISA test.

Sample pretreatment step:

A- Sample of Meat:

Two grams of a homogeneous tissue sample were weighed into the centrifuge tube. Then, 8 ml of acetonitrile solution were added to the centrifuge tube, oscillated for 2 minutes,

and then the tubes were centrifuged at room temperature at 4000 r/min for 10 minutes. Five ml Five milliliters of the supernatant were transferred into a glass tube, and the samples were blow-dried at 50-60℃ with air. One ml of redissolving solution was added to the tube and mixed thoroughly using a shaker for 30 seconds. Fifty microliters of the supernatant were used for the assay.

B- Sample of Liver:

Two grams of a homogeneous tissue sample were weighed into a centrifuge tube. Then, 8 ml of acetonitrile solution was added to the tube, oscillated for 2 minutes, and centrifuged at room temperature at 4000 r/min for 10 minutes. Five ml of the supernatant were transferred into a glass tube, and the samples were blow-dried at 50-60℃ with air. Two milliliters of N-hexane were added to the centrifuge tube and mixed with a shaker until fully dissolved. One ml of deionized water was added to the tube, mixed with a shaker for 30 seconds, and centrifuged at room temperature at 4000 r/min for 10 min. 50 µL of the lower layer was transferred to another tube, and 50 µL of the redissolving solution was added and mixed well. 50 µL of the mixed solution was used for the assay.

Analysis of samples by Ractopamine ELISA Test Kit:

(Sinogenclon Co., Ltd, Logogram: ENR Catalog No: SG-4002 Edition : 2015.1, China).

Interpretation of result:

Calculation of the percentage of absorbance value.

Percentage of absorbance value (%) = $\frac{A}{A0}$ **×100%**

The average OD value of the sample or the standard solution.

A0-the average OD value of the 0-ppb standard solution.

2.4. Drawing the standard curve and calculation:

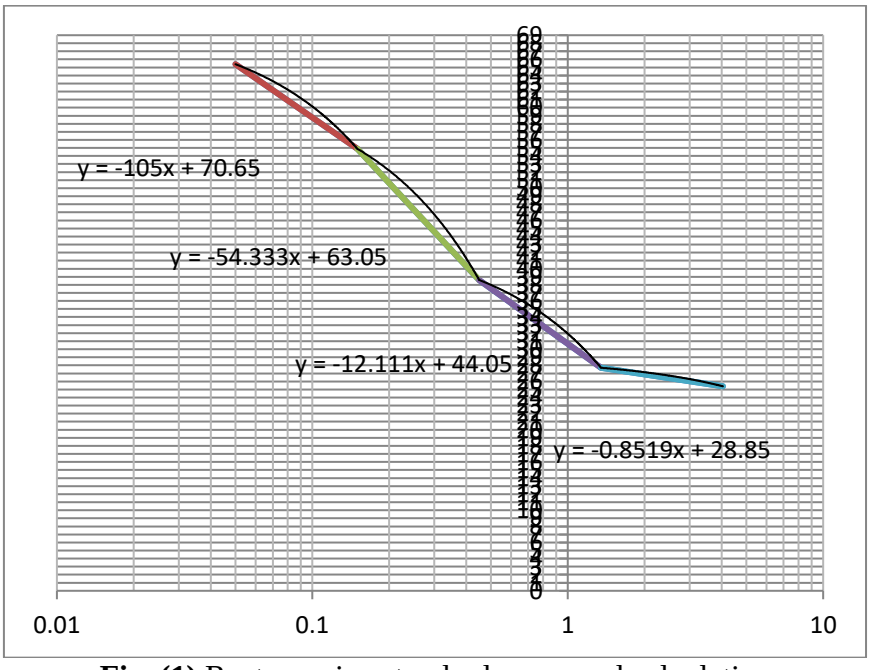

Fig. (1) Ractopamine standard curve and calculation

3. Effect of different cooking methods on Ractopamine residues in meat and liver:

Fresh local meat and liver samples were purchased from butcher shops in Assiut City, where they were analyzed to ensure they were free from RAC residues. The collected samples were transferred directly to the laboratory for preparation and cooking. The

meat samples were sliced into three equal pieces to be subjected to three different cooking treatments (boiling, grilling, and microwaving). Similarly, the liver samples were sliced into three equal pieces for three cooking treatments (frying, grilling, and microwaving). The temperature during each cooking procedure was monitored using a digital thermometer (Sky-Touch Cooking Meat Thermometer, made in Cyprus). Fresh meat and liver samples were injected with ractopamine, with each sample containing 4 ppb of ractopamine.

Heat treatment of Meat Samples:
1. Boiling at 100 °C for 120 min (Chang *et al.*, 2011).
2. Grilling at 200 ºC for 15 min (Marzouk, 2012).
3. Microwaving at 150-180°C for 8 min (Hsieh. *et al.*, 2011).

Heat treatment of liver samples:
1. Grilling at 170–200 °C/6 min (Kujawska *et al.*, 2020).
2. Frying at 230 °C for 16 min (Farag *et al.*, 1992).
3. Microwaving at 205°C for 8 min (Farag *et al.*, 1992).

4. Analysis of the heat-treated samples:
The cooked meat and liver samples analyzed by the Animal Health Research Institute consisted of 6 samples (3 samples of cooked meat and 3 samples of cooked liver). The analysis was conducted using ELISA kits from RIDASCREEN® Ractopamine, an enzyme immunoassay for the quantitative analysis of ractopamine, Art. No.: R9901, registered trademarks of R-Biopharm AG, Darmstadt, Germany. The samples were analyzed according to Food Safety Resolution No. 13 of 2020.

RESULTS

Table 1: Acceptability of the examined frozen meat samples according to the National Food Safety Authority.

Examined samples	Numbers	MRL*(ppb)	Accepted samples.		Unaccepted samples.	
			No.	**%**	**No.**	%
Meat	35	10	**33**	**94.3**	**2**	5.7

MRL*: Maximum residue limit in muscles (10ppb) according to **National Food Safety Authority (NFSA, 2020)**

Table 2: Acceptability of the examined frozen liver samples according to the National Food Safety Authority.

Examined samples	Numbers	MRL*(ppb)	Accepted samples.		Unaccepted samples.	
			No.	**%**	**No.**	%
Liver	35	20	**34**	**97**	**1**	3

MRL*: Maximum residue limit in muscles (20ppb) according to **National Food Safety Authority (NFSA, 2020)**

Table 3: Showing the effect of different cooking methods on RAC residues (4 ppb) in meat samples.

Treatment	Meat Samples	Reduction %
Boiling (100° C for 120 min.)	0.194	95.2
Grilling (200° C for 15 min.)	1.188	70.3
Microwave (180° C for 8 min.)	N. D	100

Table 4: Showing the effect of different cooking methods on RAC residues (4 ppb) in liver samples.

Treatment	Liver Samples	Reduction %
Grilling (200° C/6 min.)	0.464	88.4
Frying (230° C/16 min.)	0.409	89.8
Microwave (205° C/8 min.)	0.351	91.2

DISCUSSION

The Center for Food Safety, Center for Biological Diversity, and U.S. FDA (2014) determined that ractopamine was safe and approved by the FDA for use in cattle in 2003. No withdrawal time is required according to this FDA approval.

Regarding methodology, it is important to mention that the method used in the present study for thawing frozen imported meat samples is refrigeration at 4ºC, as recommended by Kassem-Sara *et al*. (2024), They concluded that the ideal way to thaw frozen meat is to use refrigerator thawing, as it results in less drip loss and weight loss.

From Table 1, it is evident that only two meat samples were unacceptable, representing 5.7%, while the other 33 meat samples were acceptable, representing 94.3%, according to the National Food Safety Authority (NFSA, 2020). In contrast, Jeong *et al*. (2018) reported that out of 50 imported meat samples, none contained RAC residues. Additionally, Ren and Yang (2012) emphasized that none of the 140 meat samples they examined contained RAC residues.

Data in Table 2 showed that there was only one liver sample above the MRL set by the National Food Safety Authority, representing 3%, while the remaining 34 samples were acceptable, representing 97% and below the MRL set by the NFSA in 2020. The results of the present study for RAC residues in meat samples were within the same range as those recorded by Tang *et al.* (2016). However, our results for the liver were lower than theirs. Tang et al. found that the concentration of RAC residues in liver and meat samples collected from 6 animals on both withdrawal day 0 and day 3 were 119.41 and 10.88 ppb for liver samples, and 4.17 and 1.13 ppb for meat samples.

In general, imported frozen liver should be consumed in moderation at intervals to avoid potential health problems (Ahmed *et al*., 2020).

From Table 3, it is evident that the reduction percentage of RAC after different cooking methods of meat samples was 95.2% in the

case of boiling (at 100°C for 120 minutes), 70.3% in the case of grilling (at 200°C for 15 minutes), and 100% in the case of microwaving (at 180°C for 8 minutes). The results of the present study revealed that the most effective cooking methods for reducing or eliminating RAC hormone levels in beef muscles were microwaving, followed by boiling, and then grilling. In a related study, Chang *et al*. (2011) found that the mean percentage of degradation of RAC residues in beef muscle was 17.9% after boiling at 100°C for 60 minutes. On the contrary, Rose *et al*. (1995) mentioned that β-agonist residues were stable after boiling in water at 100°C for 5 hours. Hassan *et al*. (2016) recorded that the reduction percentage of boiling meat samples at 100°C for 60 minutes was 19.24%, which is lower than the obtained result.

The results in Table 4 showed that in the case of grilling (at 200°C for 6 minutes) of beef liver samples, the reduction percentage was 88.4%. Meanwhile, in the case of frying (at 230°C for 16 minutes), the percentage was 89.8%, and in the case of microwave cooking (at 205°C for 8 minutes), the reduction percentage was 91.2%. These results revealed that the most effective cooking methods for reducing or eliminating RAC hormone levels in beef liver were microwaving, followed by frying, and then grilling.

While there are no current food safety concerns with ractopamine in our meat supply, you may wish to follow these tips for further reassurance and to reduce your exposure to ractopamine in meat:

1. Cook meat thoroughly before consuming it, as heat can aid in breaking down any remaining ractopamine:
- To ensure that imported frozen beef is cooked thoroughly, it must be boiled for 120 minutes at 100°C, grilled for 15 minutes at 200°C, or microwaved for 8 minutes on high power.
- To ensure that imported liver is cooked thoroughly, it must be grilled for 6 minutes at 170-200°C, fried for 16 minutes at 230°C, or microwaved for 8 minutes on high power.

2. The optimum cooking technique for reducing RAC residues in beef liver and muscles is microwaving.

3. Reduce consumption of organs such as imported frozen liver, which may contain relatively higher levels of ractopamine.

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التحكم في متبقيات الراكتوبامين في اللحوم والكبد المجمدة المستوردة باستخدام طرق طهي مختلفة

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أجريت هذه الدراسة للكشف عن متبقيات الراكتوبامين في ٧٠ عينة من اللحوم والكبد المجمدة المستوردة بواقع ٣٥ عينة من كل نوع ، وتم جمع العينات من متاجر اللحوم المجمدة المختلفة بمدينة أسيوط وكذلك من المدن الجامعية لجامعة أسيوط. وقد أشارت نتائج بقايا الراكتوبامين إلى أن ٩٤,٣ ٪ من عينات لحوم البقر المجمدة المستوردة تم قبولها، و بنسبة ٥، ٧٪ غير مقبولة ، وكانت أعلى من الحد الأقصى لمخلفات الراكتوبامين الذي حددته هيئة سلامة الغذاء المصرية. وفيما يتعلق بالكبد المجمد فقد تم قبول ٩٧٪ من العينات وعدم قبول ٣٪ طبقا لهيئة سلامة الغذاء المصرية. وبهذا؛ فإن كمية بقايا هرمون الراكتوبامين المكتشفة في اللحوم والكبد المجمدة المستوردة تعد آمنة. علاوة على ذلك فقد تم تطبيق طرق طهي مختلفة على عينات من اللحوم وذلك بإضافة تركيز معروف من الراكتوبامين (٤ جزء في البليون) للكشف عن تأثير المعالجة الحرارية على بقايا الراكتوبامين. وقد تم ذلك بطهي بعض عينات اللحوم بالغليان عند درجة حرارة ١٠٠ درجة مئوية لمدة ١٢٠ دقيقة، وأخرى للشوي عند درجة حرارة ٢٠٠ درجة مئوية لمدة ١٥ دقيقة، وللميكروويف عند درجة حرارة ١٥٠-١٨٠ درجة مئوية لمدة ٨ دقائق. أما عن عينات الكبد فتم طهيها عن طريق الشوي عند درجة حرارة ١٧٠-٢٠٠ درجة مئوية / ٦ دقائق، وكذلك القلي عند ٢٣٠ درجة مئوية لمدة ١٦ دقيقة، والميكروويف عند ٢٠٥ درجة مئوية لمدة ٨ دقائق. وكانت طرق الطهي الأكثر فعالية لتقليل بقايا الراكتوبامين هي الميكروويف يتبعها الغليان والقلي ثم الشوي.