



## Effect of different cooking methods on ractopamine residues in beef

Mohammed, A. Hassan, Reham, A.A.<sup>1</sup>, Marzouk, N. M. and Sarah, R.kh.<sup>2</sup>

<sup>1</sup> Food Control Department, Faculty of Veterinary Medicine, Benha University.

<sup>2</sup> Food Hygiene Department, Animal Health Research Institute, Dokki, Giza.

### ABSTRACT

The study was planned out to estimate the ractopamine (RAC) residues in frozen beef as well as the effect of different cooking on (RAC) in beef samples. The mean value of RAC in meat was  $3.43 \pm 0.43$  ppb. The obtained results showed no evidence for the illegal use of ractopamine, but these results do not exclude the possibility of misuse of these potentially harmful Substance. Regarding the effect of cooking on RAC residues in different cooking processes (boiling, microwaving and grilling), results showed that the degradation of RAC after boiling was 19.24%. Whereas after microwaving and grilling they were 11.66, 47.52%. The results were statistically evaluated and the public health significances were discussed.

**Key words:** Ractopamine, Beef, Boiling, Microwave, Grilling

(<http://www.bvmj.bu.edu.eg>)

(BVMJ-31(2): 210-212, 2016)

### 1. INTRODUCTION

Meat makes up a considerable portion of a typical diet. It contributes protein, minerals, vitamins and fat, and these nutrients are important for their beneficial effects on well-being. Ractopamine is administered to animals as feed additives, and the most serious potential hazards arising from using ractopamine are the tissue residues of this substance. Ractopamine is defined as a synthetic substance that is used as a veterinary drug in animal feed to promote muscle growth in approved food animal species, namely pigs, cattle and to a limited extent, heavy turkeys (FAO, 2012). Ractopamine is a beta-adrenergic agonist widely used in diets for finishing animal by providing a redirection of nutrients, reducing fat and increasing lean meat deposition in the carcass. It also promotes improved in performance and carcass characteristics of animals (Campos et al., 2013). Ractopamine have been illegally used to promote protein synthesis and to increase the feed conversion rate in livestock. However, the residue of ractopamine could cause potential hazards for human health (Li et al., 2015). Ractopamine, a beta-adrenergic leanness-enhancing agent, endangers the food safety of animal products because of overdosing and illegal use in food animals (Huang et al., 2016) Ractopamine residues in humans can cause poisoning and the consumption of meat and/or meat products derived

from animals that used ractopamine in feed for growth stimulation can suffering from tachycardia, headache, muscle spasm, high blood pressure, muscle tremor, restlessness, apprehension, anxiety (EFSA, 2009). The permissible limit of ractopamine residues for muscle, liver, and kidneys were  $10.0 \mu\text{g}/\text{kg}$ ,  $40.0 \mu\text{g}/\text{kg}$  and  $90.0 \mu\text{g}/\text{kg}$ , respectively. (Codex, 2012).

Thus, this work was done to determine the level of ractopamine residues in beef with reduction effect by different cooking methods.

### 2. MATERIAL AND METHODS

#### 2.1. Collection of samples:

A grand total of 40 samples of frozen beef divided into 4 groups (10 of each), the first group subjected to analysis for detection of ractopamine residues and other three groups were subjected to experimental three different heat treatments, boiling at  $100^\circ\text{C}$  for 120 min (Chang et al., 2011), grilled at 200 for 15 min (Marzouk, 2012) and microwaved at  $150\text{-}180^\circ\text{C}$  for 8 min. (Hsieh. et al., 2011) according to manual kits ELISA (R-Biopharm AG, Darmstadt, Germany).

#### 2.2. Preparation of samples:

The fat was removed and the sample was homogenized to a fine mass, using stomacher or

mixer. Three grams of the homogenized sample were transferred into a centrifugal screw cap vial, 8 ml of acetonitrile and 1ml of ethyl acetate were added and mixed well using vortex. Shaking for 30 min (with up-side-down shaker). Then Centrifuge for 10 min at 4000 rpm. (20-25°C). 4 ml of supernatant (corresponding to 1 gm of sample) were transferred into a new centrifugal vial and evaporated to dryness at 60°C. The residue was dissolved in a 2-ml n-hexane (or n- heptane) and 1 ml of sample dilution buffer and vortex for 30 second. Then Centrifuge for 10 min at 4000 rpm. (20-25°C). The lower phase was removed carefully. Then 20 µl of the lower phase was used per well in the test. Test procedures: The test procedures were done according to the chart enclosed in the kits of RIDA® and RIDASCREEN® is register trademarks of R-Biopharm AG. Manufacture: R-Biopharm AG, Darmstadt, Germany. R-Biopharm AG is ISO certified.

2.3. Detection of ractopamine in beef samples.

2.4. Effect of boiling on beef at 100°C for 60 min, grilled at 200 for 15 min and microwaved at 150-180°C for 8 min.

3. RESULTS

In order to obtain the ractopamine concentration in (µg/kg) ppb actually contained in the samples. The concentration was read from the calibration standard curve (Fig. A). The result was calculated by this equation: % absorbance = (OD sample/ OD standard) x 100, results were calculated as (µg/kg). Table (1) revealed that the mean values of ractopamine residue in control beef sample were 3.43±0.43ppb with significant difference with ractopamine residue after boiling and grilling, but with no significant difference with ractopamine residue after microwaving.

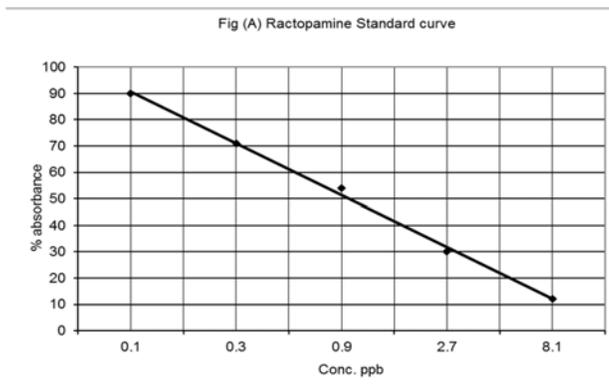
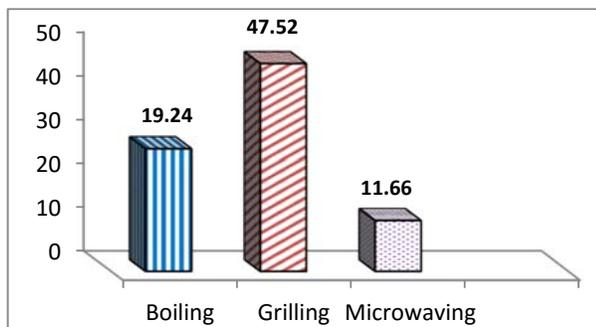


Table (1): Effect of different cooking methods on ractopamine residues (ppb) in beef samples (n=10)

Item	Minimum	Maximum	Mean ± SE	Reduction%
Control	1.90	5.00	3.43±0.43 <sup>a</sup>	-
Boiling	1.54	4.00	2.77±0.35 <sup>b</sup>	19.24
Grilling	1.00	2.60	1.80±0.23 <sup>c</sup>	47.52
Microwaving	1.70	4.40	3.03±0.39 <sup>a</sup>	11.66

Different letters within the same column represent significance differences (P<0.05).

Figure (B): Effect of different cooking methods on ractopamine residues (ppb) in beef samples.



#### 4. DISCUSSION

In the present study, the ELISA method was used to identify ractopamine residues in meat. Recently, Enzyme-Linked Immunosorbent Assays (ELISA) have been established as screening methods for ractopamine residues in imported and exported meat (Chai et al., 2013). According to the results in Table (1) the mean values of ractopamine in beef samples before cooking method was  $3.43 \pm 0.43$   $\mu\text{g}/\text{kg}$  with minimum and maximum values of 1.90 and 5.00 ppb. The results illustrated the effect of boiling on ractopamine residues in meat samples at  $100^\circ\text{C}$  for 60 minutes was reduced to  $2.77 \pm 0.35$  ppb. There were significance differences ( $P < 0.05$ ) between ractopamine residue in original raw beef sample and its residue in heat treated meat samples at  $100^\circ\text{C}$  for 60 minutes with reduction rate 19.24 %. Similar results were reported by (Chang et al., 2011) who found nearly mean percentage of degradation of ractopamine residues in beef tissue (muscle) which was 17.9 % after boiling at  $100^\circ\text{C}$  for 60 minutes. Unlike (Rose et al., 1995) who stated that  $\beta$ -agonist residues was stable after boiling in water at  $100^\circ\text{C}$ . Also, the effect of different cooking methods on ractopamine residues in beef, the level of ractopamine residue in meat tissue before grilling was  $3.43 \pm 0.43$  ppb, but after grilling the ractopamine residues decreased with reduction percent 47.52 % to become  $1.80 \pm 0.23$  ppb. There was significance difference ( $P < 0.05$ ) between ractopamine residues before and after grilling. Moreover, by microwaving, the level of ractopamine residues decreased with reduction percent of 11.66% to become  $3.03 \pm 0.39$  ppb. There was no significance difference between ractopamine residues before and after microwaving. Also, there was significance difference ( $P < 0.05$ ) between ractopamine residues after microwaving and grilling method for cooking. The result of grilling agree with those of (Sudchada, 2005) who showed that  $\beta$ -agonist residues as growth promoters decreased by 50% after roasting at  $200^\circ\text{C}$  for about 10 minutes. On contrary, (Marzouk, 2012) showed the reduction percent of ractopamine residues after barbecued at  $200^\circ\text{C}$  for 15 minutes, was 99.88%. The results of microwaving agree with those of (Hsieh. et al., 2011) who showed that the degradation of ractopamine in water was highest after autoclaving (13.1%) and lowest after microwaving. Therefore, it is suggested that grilling can be used as the best method of cooking in reducing ractopamine residues in beef.

#### 5. REFERENCES

- Campos, P.F., Scotta, B.A., Oliveira, B.L., 2013. Influence of ractopamine in meat quality of pigs. *Revista Brasileira de Agropecuaria Sustentavel*. 3, 164-172.
- Chai, MingJun., Hui, HongWen., Huang, C., 2013. Ractopamine detection in meat and meat products by ELISA. *Food Research and Development*. 34, 78-80.
- Chang, ShaoKuang., Hsieh, MingKun, Lee, WeiMing, Lan, PeiYu, Zhang, WenFa, Chou, C., 2011. Evaluation of the heat stability of ractopamine in beef, pork, swine liver, swine kidney and swine lung. *Taiwan Veterinary Journal*. 37, 253-261.
- Codex, A.C., 2012. Joint FAO/WHO Standards Programme Codex Alimentarius Commission, Thirty-fifth Session FAO Headquarters, Rome, Italy. 2-7 July 2012 Report. REP12/CAC, Rome. FAO/WHO, Rome, Italy.
- EFSA, 2009. (European Food Safety Authority) Scientific Opinion, Safety evaluation of ractopamine 1, Scientific Opinion of the panel on Additives and products or Substances used in animal feed. . *EFSA Journal* 1041.
- FAO, 2012. (Food and Agriculture Organization of the United Nations ). Information sheet - Discussion on ractopamine in codex and in the Joint FAO/WHO Expert Committee on Food Additives (JECFA).FAO, Rome, Italy, 1-2.
- Hsieh., MingKun., Chang., ShaoKuang., Lan, P., Chou, C., 2011. Studies of heat stability of ractopamine in water and soy sauce. . *Taiwan Veterinary Journal*. 37, 111-118.
- Huang, L., Shi, J., Pan, Y., Wang, L., Chen, D., Xie, S., Liu, Z., Yuan, Z., 2016. Elimination and Concentration Correlations between Edible Tissues and Biological Fluids and Hair of Ractopamine in Pigs and Goats Fed with Ractopamine-Medicated Feed. *Journal of agricultural and food chemistry* 64, 2012-2020.
- Li, C., Li, J., Jiang, W., Zhang, S., Shen, J., Wen, K., Wang, Z., 2015. Development and Application of a Gel-Based Immunoassay for the Rapid Screening of Salbutamol and Ractopamine Residues in Pork. *Journal of agricultural and food chemistry* 63, 10556-10561.
- Marzouk, N.M., 2012. Effect of Different Heat Treatment on Ractopamine Residue in Cattle's Meat, Liver and Kidney. . *J. of Appl. Sci.* 27(12B): 297-307.
- Rose, M.D., Shearer, G., Farrington, W.H., 1995. The effect of cooking on veterinary drug residues in food: 1.Clenbuterol. *Food Addit. Contam.* 12, 67-76.
- Sudchada, S., 2005. Effect of Heating on salbutamol Residue in Raw pork Tissue. *Bulletin of the Department of Medical Sciences*. 47, 1-13.