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# ANALYSIS OF RACTOPAMINE RESIDUES IN COMMERCIAL SWINE SAMPLES OF KIDNEY, LIVER AND LUNG

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## Introduction

Pork is the most consumed meat in the world (OURIQUE; NICOLAIEWSKY, 1990). Therefore, methods are constantly being sought to ensure food safety and increase pork quality production. In order to accomplish meat quality there are genetic and nutritional improvements, such as the use of additives in animal feed (LEAL et al., 2014).

Additives have been used to modify metabolism in several species. Among these substances, Ractopamine hydrochloride (RAC), a β-adrenergic agonist derivative with a structure analogous to catecholamines (Apple et al., 2007) has been added to animal feed up to 20 mg $kg^{-1}$. It acts in energy metabolism, redirecting nutrients from the diet, promoting lipolysis and muscular hypertrophy (GUNAWAN et al., 2007; WATKINS et al., 1990).

Used in finishing diets of pigs (28 days), RAC propitiates better feed conversion (about 12%) and consequently increases average daily weight gain and final weight (SANCHES, 2009). Studies indicate that meat quality is not affected by the addition of RAC in animal feed (WARRIS et al., 1990; ZAGURY et al., 2002).

Despite the many benefits of RAC, the *Codex Alimentarius* has established the maximum residue limits (MRLs) of 90, 40, 10 and 10 μg $kg^{-1}$ for kidney, liver, fat, and pork, respectively, as recommended by the Joint Expert Committee on Food Additives (JECFA, 2014). Although *Codex Alimentarius* set these limits, each country has its own standards. Countries such as China, Taiwan and even the European Union have zero tolerance policies for RAC residues in pork products (CENTNER; ALVEY; STELZLENI, 2014).

Therefore, the objectives of this research were to validate an analytical method to determine ractopamine residue in pig kidney, liver and lungs and apply this validated method to real samples. The method performance was evaluated through specificity, recovery, linearity, reproducibility (inter-day precision), repeatability (intra-day precision), decision limit ($CC_\alpha$), and detection capability ($CC_\beta$), in accordance to the Commission Decision 2002/657/EC.

## Material and methods

### Sample collection

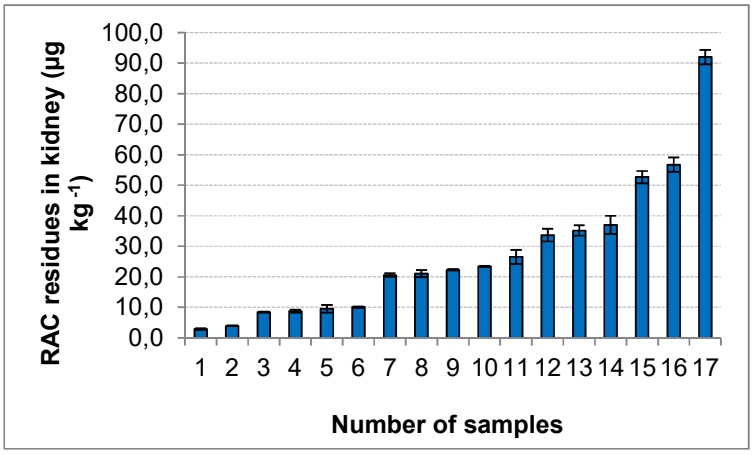
We collected 17 commercial samples for each of the organs at slaughter: kidney, liver and lungs. Initially, the matrices were cut and homogenized, after weighing 5.00 g of sample. The internal standard (IS) was added at a concentration of 10 μg $kg^{-1}$. In order to extract RAC from samples by QuEChERS, they were digested with β-

glucuronidase enzyme in a water bath, then cooled and had their pH adjusted. The enzyme was added to release RAC from bounded glucuronides. Afterward, sample was submitted to cleaning, centrifugation and then finally transferred to a flask for LC-MS/MS analysis.

## Results and discussion

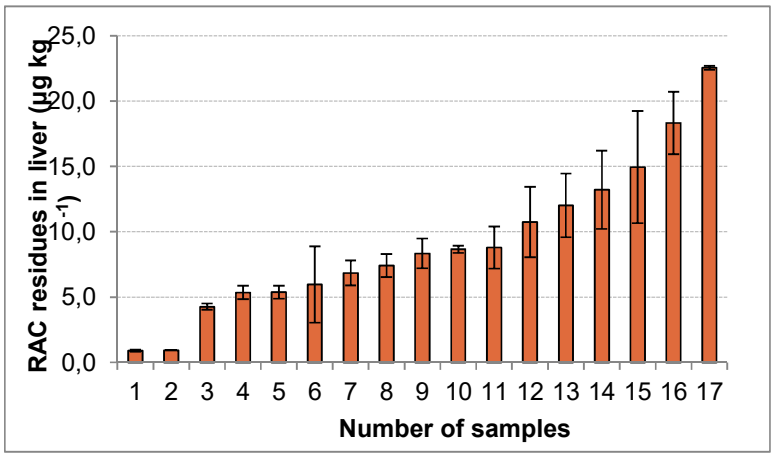
The concentrations of RAC residues in kidney, liver and lungs are shown in Graphs 1, 2 and 3. The values (μg $kg^{-1}$ ± SD) ranged from 2.9 ± 0.2 to 92 ± 2 for kidneys, 0.9 ± 0.1 to 22.5 ± 0.1 for liver and < CCβ and 1003 ± 45 for lungs.

**Graph 1 - Concentrations of ractopamine residues in kidney samples.**




Limit of detection = 0.5 μg $kg^{-1}$; Limit of quantification = 2.5 μg $kg^{-1}$; $R^2$ = 0.996; $CC_\alpha$ = 4.39 μg $kg^{-1}$; $CC_\beta$ = 6.27 μg $kg^{-1}$; Recovery = 92–98%; Intra-day = 7.4–15.8%; Inter-day = 5–17.9%; Matrix effect = - 57.9%.

**Grap h 2 -**




**Concentrations of ractopamine residues in liver samples.**

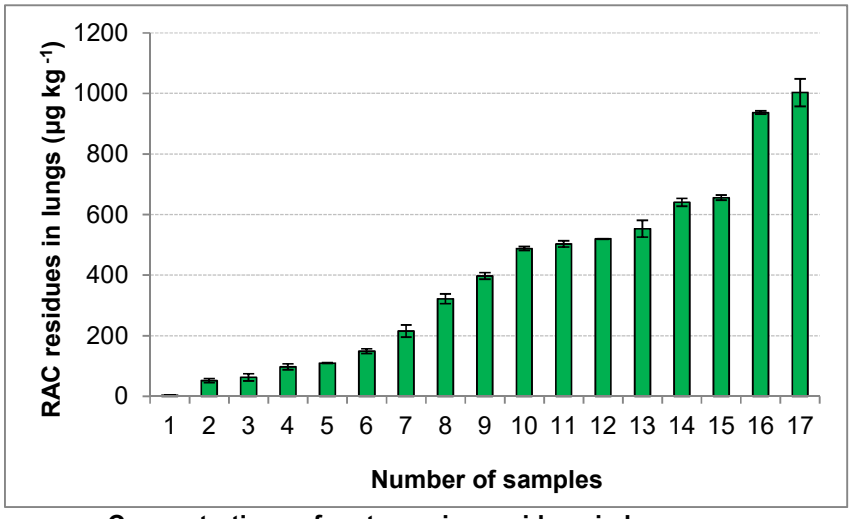
**Figure 2 -** LD- 0,5; LQ- 2,5.

Limit of detection = 0.5 µg $kg^{-1}$; Limit of quantification = 2.5 µg $kg^{-1}$; $R^2$ = 0.999;
$CC_\alpha$ = 4.86 µg $kg^{-1}$; $CC_\beta$ = 7.21 µg $kg^{-1}$; Recovery = 96–98%;
Intra-day = 5.7–14.2%; Inter-day = 7.8–16.4%; Matrix effect = - 54.4%.

It can be observed that only one kidney sample (17) overcame the MRL of 90 µg $kg^{-1}$ of RAC residues. Thus, it is possible to see the efficiency of the inspection systems and the commitment of the agroindustrial sector to the quality of meat intended for marketing. Kidneys have a food importance in some countries around the world, especially in Southeast Asia (USDA, 2016).

It was observed after analyzing liver samples collected in commercial slaughter houses that all samples were in agreement with the MRL of RAC residues in liver of 40 µg $kg^{-1}$ established by the *Codex Alimentarius*.

**Graph 3 -**




**Concentrations of ractopamine residues in lungs.**

The lungs were the organs with the highest concentrations of RAC compared to other analyzed tissues. Similar results were found by Dong et al. (2011), which observed the following descendent concentrations in a variety of

Limit of detection = 1.0 µg $kg^{-1}$; Limit of quantification = 2.5 µg $kg^{-1}$; $R^2$ = 0.999;
$CC_\alpha$ = 3.65 µg $kg^{-1}$; $CC_\beta$ = 7.05 µg $kg^{-1}$; Recovery = 94–127%; Intra-day = 16.5–24.1%;
Inter-day = 6.7–22.3%; Matrix effect = - 69.9%.

matrices: lung (599 μg $kg^{-1}$) > kidney > small intestine > large intestine > stomach > liver > hearth > muscle.

These results may be due to the high number of β-agonists receptors present in lungs. For instance, pigs snout is a very effective organ for exploring the environment by rooting, sniffing, chewing and manipulating; thus inhaling RAC particles from feed is possible (VAARST; LUND; RODERICK; LOCKERETZ, 2004).

Despite the high concentration of RAC in this sample, there are no restrictions established by *Codex Alimentarius* regarding the maximum limit of this contaminant. In addition, the lung is a by-product consumed especially in Asian countries and may become harmful to these consumers, when the concentration of RAC is very high, because it is a β-adrenergic agonist capable of causing tachycardia (EFSA, 2009).

## Conclusion

The RAC additive is widely used in pig production in several countries, while other impose a strong restriction in its use. It was observed that among the analyzed samples of kidney, liver and lungs only one sample was over the recommended MRL suggested by the *Codex Alimentarius*. In addition, it is necessary to establish a MRL for lung samples, because it is an organ largely consumed in Asian countries and may cause health problems to humans when consumed in high concentration.

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