The challenges of ractopamine use in meat production for export to European Union and Russia

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A B S T R A C T
β-adrenergic agonist ractopamine is increasingly used in the swine industry due to consumer demand for leaner pork products. Ractopamine redirects nutrients to favor leanness rather than fat deposition, improves growth and carcass traits of finishing pigs. However, countries around the world are split over whether to allow the use of ractopamine in meat production. While this substance has been authorized as a feed additive in a limited number of countries, in pigs and cattle, the vast majority of jurisdictions, including the European Union (EU), China, Taiwan, Russia has banned its use on safety grounds. This legal division finds reflection into the long-standing opposition existing between countries supporting the establishment of maximum residue levels and those who oppose it within the Codex. In fact, the international debate over ractopamine bans, restrictions and maximum residue level standards have intensified and a trade war may be looming. A conflicting debate between countries has started. In this article, we discuss about the criteria of “level and not a limit”, comprehensive methods used for ractopamine monitoring on every stage of the production chain, and the recommended tissue for analysis.

1. Introduction
β-adrenergic agonists (β-agonists) are widely used as bronchodilators, tocolytics and heart tonics in clinical and veterinary medicine (Antignac, Marchand, Le Bizec, & Andre, 2002). Ractopamine is classified as a β-adrenergic agonist that acts as a reprogramming agent, redirecting nutrients away from adipose tissue towards muscle deposition, resulting in substantial improvements in average daily gain, feed conversion efficiency, dressing percent and carcass lean content (Gu, Schinckel, Forrest, Kuei, & Watkins, 1991; Rikard-Bell et al., 2009). A reduction in the deposition of adipose tissue in the carcass of pigs fed the diets containing ractopamine occurs through two metabolic pathways: reduction in lipogenesis and/or increase in lipolysis. Analysis of the adipocytes isolated from these fed pigs, (Liu, Boyer, & Mills, 1989) reported lipolytic rate of these cells through the formation of glycerol and found that the reduction in adipose tissue in the carcass occurred through an increase in lipolysis. On the other hand, studies in vitro, in swine adipocytes measuring the rate of lipogenesis through the incorporation of glucose labeled with 14C in fatty acids resulted a reduction in lipogenesis (Mills & Liu, 1990; Peterla & Scanes, 1990).

Countries around the world are split over whether to allow the use of ractopamine in meat production. Ractopamine is banned or restricted in 160 countries, including China, Russia, and members of the European Union (EU) while 27 other countries, such as Japan, the United States, Canada, Brazil and South Korea, have deemed meat from livestock fed ractopamine safe for human consumption (Pacelle, 2014).

After years of scientific and political deadlock, the Codex Alimentarius Commission, on July 5, 2012 adopted the first-ever maximum residue levels (MRL) for ractopamine hydrochloride as 10 ppb for beef and pork. This decision was a big win for the United States’, Brazil’s and Canada’s trade interest and loss for more than 100 countries that had banned ractopamine. Brazil, one of the major beef exporters into the EU and other countries, authorized the use of ractopamine hydrochloride, in swine, in June 2012 and immediately informed the Eurasian Customs Union (ECU), an association between Russia, Kazakhstan and Belarus to maintain the pork meat export authorization. ECU communicated to Brazil that is mandatory to put in place a “split system” and to immediately...
notify this to the World Trade Organization (WTO) if the country wishes to continue export to ECU. Failing to do so, the ECU will have to stop, without exception, the import of pork meat and other swine products from Brazil. In fact, by the end of 2012, Brazil started to implement organizing production segregation process in order to comply with the demands of its largest meat importer, the ECU.

In January 2013, Russian banned U.S. pork, chicken and beef imports due to use of controversial ractopamine. U.S. interests believe that the ban was more related to political issues between the U.S. and Russia than the issue of ractopamine. In response to the new policy on ractopamine, Canada and Brazil reportedly gave Russian authorities assurance that pork and beef exports were certified ractopamine-free before being shipped to Russia. The European Union Council Directive 96/22/EC, concerning the prohibition of certain drugs and hormones in livestock production, is a strong legislation applied to member states and to third countries (European Commission, 1996). A split system to ensure animals not treated at any stage in production should be in place to ensure eligibility of the products for export to EU. If residues of this class of drugs are found on Rapid Alert System for Foods and Feeds (RASFF), in concentrations above minimum performance required level (MPRL) and level of action (RPA) will lead to either product rejection, recall, product decontamination and/or protective measures which involve intensive testing, and even country delisting. For these reasons, it is important to develop methods, and also to make a decision on which tissue to test for ractopamine. Some governments have established levels of action in order to guarantee that the meat consignments sent out to those countries have a low/no risk of containing ractopamine residues. The ECU also established an action level for 0.1 ppb of ractopamine on beef consignments sent to this common market, and attention is demanded that it is a “level and not a limit”. This means that if a laboratory found a confirmed concentration of less than 0.1 ppb, the consignment must be rejected. The EU has set a level of action of 1 ppb in urine and the same criteria of “level and limit” should be considered. Methods applied to EU monitoring should be capable to quantify ractopamine levels of 0.17 ppb.

In this short review we discuss the two criteria and present which of them indicates lower risks for the presence of ractopamine residues. Additionally, considering the total ban of ractopamine from important markets in which Brazil exports meat consignments, the objectives of this paper are to address: a) the need of ractopamine monitoring on every stage of the production chain; b) the methods used for ractopamine monitoring and the risks involved on the choice, and c) the risk involved in monitoring different tissues.

2. Analytical methods

2.1. ELISA assay

Several screening methods for ractopamine have been reported based on enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) (Elliot, 1998; Shaveler & Smith, 2000; Wang, Li, Zhang, & Shen, 2006; Wang, Zhang, & Shen, 2006). The detection limits of these methods are in the 0.1–50 ng/mL (ppb) range (Dong et al., 2012; Shaveler & Smith, 2002; Shen et al., 2007) in different tissues. These methods are able to detect ractopamine without complicated purification, due to the specificity of antibody used, though matrix effects often occur. However, these methods each have drawbacks, especially for use in developing countries and are very expensive considering shipping and importation costs. Methods based on ELISA have been shown to be frequently prone to poor performance in developing countries, probably due to degradation of reagents (especially the enzyme-conjugate) under less than ideal conditions during transport and storage (Cannavan & Elliott, 2004; IAEA, 2003). Another interesting point regarding ELISA technique for ractopamine is the fact that it is not clear to the users the need of a deconjugation step for the method, to allow the kit to detect the total ractopamine and not only free ractopamine. Ractopamine is a β-agonist that conjugates with glucuronic acid, once administered to the animals.

2.2. Use of GC-MS, LC-MS and UPLC-MS

Other analytical methods have been reported for the determination of ractopamine in biological samples, including gas chromatography—mass spectrometry (Wang, Li, et al., 2006; Wang, Zhang, et al., 2006), liquid chromatography—mass spectrometry (Pleadin, Vulic, Persi, & Radeck, 2011; Sakai et al., 2007; Shishani, Chai, Jamokha, Aznar, & Hoffman, 2003). However, these methods not only require tedious pre-treatment procedures but also use expensive instrumentation (Qu et al., 2011). So far, ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) is one of the most efficient methods (Shao et al., 2009) because of the high resolution, rapid separation of UPLC, and the selectivity and sensitivity characteristic of MS/MS detection (Dong et al., 2012). Although requires expensive sample preparation methodology. A validated UPLC-MS/MS method is available for determination of ractopamine in pig urine and hair at trace levels (Pleadin, Vulic, Persi, & Radeck, 2012). The method performance characteristics were found to be in accordance with the EU requirements and its suitability for determination of low ractopamine residues in real samples. Indeed, since ractopamine is a banned drug in a lot of countries, there is a “zero tolerance” factor involved on the decisions, and the detection levels of the immunoassay screening kits available on market are very close to the action level determined by ECU, resulting in a considerable risk factor involved. The decision of sending swine meat to ECU based only on screening test kit results may not guarantee that the swine meat consignments is not positive for ractopamine residues.

3. Feed monitoring

When ractopamine is added to feed and/or administered to animals, it is distributed via the blood to the muscle tissues, where it bind to specific β-receptors in the muscle cell membranes. This triggers a cascade of events that initiates an increase in protein synthesis, which in turn results in increase in muscle fiber size. The substance is readily absorbed, distributed and eliminated via urine and faeces. It is intended only for use in mature (finishing) animals (pigs greater than 110 kg) prior to slaughter (Apple et al., 2007).

Feed production is a very complicated process and implementing a segregate line of production can be a problem. For instance, feed manufacturers which produce feed containing ractopamine and feed that does not contain ractopamine must have in place a verifiable Hazard Analysis and Critical Control Points (HACCP) program which will include Standard Operating Procedures (SOPs) for the prevention of ractopamine cross contamination. This includes sequencing and flushing procedures for all equipment used in the manufacturing of feed and the delivery of feed or the use of segregated equipment from load-in to delivery. This is because there is not a specific guideline on the requirements and how to assemble a segregated line following those requirements, especially by import countries. On the other hand, the same importers will look for evidence and outcomes of this implementation. In addition there is the need of certifying the segregation from the birth of the animal to its meat-processing step at the end of the chain and therefore every stage of the process need to be monitored. A special attention should be drawn to the possibility of...
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