Quality of salted sardine (*sardina pilchardus*)

Abstract

Fish has always been extremely important in human nutrition due to its culinary and nutritional values. Throughout history, different methods of conserving fish have been developed in order to preserve its quality, the best known being curing in salt. The objective of this study was to describe the production technology of salted sardines harvested from the open seas, and to determine the chemical composition and quality of the finished product. Analyses were performed on samples of salted sardines taken from a 5-kilo can. Fish were sampled randomly from the entire content of the can. In the sensory analysis, all examined samples of fish were of satisfactory quality. The analysis of the fish abdominal cavity and muscles found no parasites. The mean value of fish mass was 22.97 g. The average amount of water was 46.61%, 8.92% of fat, 8.40% of protein and the analysed samples contained 22.54% of salt. The results indicate that the irreproachable sensory properties of salted sardines depend on the proper handling of fish after harvesting as well as on the processing methods. To obtain this traditional product, it is necessary to use high quality raw material (fresh fish of impeccable quality), cure it in pure salt in proper sequence, store it in appropriate conditions and check it regularly.

Key words: *salt, salted sardines, quality, chemical composition*

Qualität der Salzsardinen (*Sardina pilchardus*)

Zusammenfassung

Fisch war immer außerordentlich wichtig in menschlicher Ernährung, dies dank seiner gastronomischen und nutritiven Werten. In der Geschichte wurden verschiedene Konservierungsarten für Fisch entwickelt, um die Qualität der Fische zu bewahren. Die bekanntesteArt ist das Einsalzen. Das Ziel dieser Arbeit wäre, die Herstellungstechnologie der eigesalzenen Sardinen zu beschreiben, die im offenen Meer eingefangen wurden, sowie die chemische Zusammensetzung und die Qualität des Endproduktes zu bestimmen. Die Analysen wurden auf Mustern von eingesalzenen Sardinen aus der 5 kg Dosen, durchgeführt. Die Fische sind zu Musterprobe durch die Methode der zufälligen Auswahl aus dem Gesamtinhalt der Dose genommen. Gelegentlich der sensorischen Untersuchung waren alle Fische von zufriedenstellender Qualität. Bei der Untersuchung der Bauchhölle und der Muskeln wurden keine Parasiten vorgefunden. Der Mittelwert der Fischmasse betrug 22,97 g. Die durchschnittliche Menge betrug: Wasser 46,61 %, Fett 8,92 %, Eiweißstoffe 8,40 %. In den untersuchten Mustern wurde die Menge von 22,54 % Salz bestimmt. Die bekommenen Resultate weisen darauf hin, dass die Vollkommenheit der sensorischen Eigenschaften der eingesalzenen Fische von der richtigen Behandlung der Fische nach dem Fang und von der Bearbeitungsmethode abhängt. Um dieses autochthone Erzeugnis zu gewinnen ist es nötig, qualitativ gute Rohstoffe (frische Fische von bester Qualität) zu verwenden, das reine Salz ordnungsgemäß beizugeben, geeignet zu lagern und regelmäßig zu kontrollieren.

Schlüsselwörter: *Einsalzen, Salzsardinen, Qualität, chemische Zusammensetzung*

La calidad de sardina salada (*Sardina pilchardus*)

Resúmen

El pescado siempre ha constituido un papel importante de la dieta humana por sus características gastronómicas y valores nutritivos. Durante la historia se usaron diferentes métodos de conservación del pescado con el fin de conservar su calidad, y el método más conocido es la salazón. El objetivo de este artículo fue describir la tecnología de la producción de las sardinas saladas cogidas en la alta mar y determinar la composición química y la calidad del producto final. Los análisis fueron hechos en las muestras de la sardina salada de las hojalatas del volumen de 5 kg. El pescado fue muestreado por el método aleatorio del contenido total de la hojalata. El análisis sensorial mostró que todas las muestras son de calidad satisfactoria. Durante la revisión de la cavidad abdominal y la musculatura del pescado no fueron encontrados los parásitos. El valor medio del pescado fue 22,97 g. La cantidad promedia del agua fue 46,61%, de grasa 8,92% y de proteína 8,40%. En las muestras analizadas fue detectado 22,54% de sal. Los resultados obtenidos apuntan que las calidades sensoriales perfectas de la sardina salada dependen del manejo apropiado del pescado después de la pesca y del método de la elaboración. Para obtener este producto autóctono es necesario usar materia prima de calidad (el pescado fresco de calidad impecable), hacer la secuencia correcta del salazón con la sal pura, almacenarlo en las condiciones apropiadas y controlar regularmente.

Palabras claves: *salazón, sardinas saladas, calidad, composición química*

la qualità delle sardine (*Sardina pilchardus*) sotto sale

Sunto

Il pesce, grazie alle sue proprietà gastronomiche e nutrizionali, è sempre stato molto importante nell'alimentazione umana. Nel corso dei secoli l'uomo ha sviluppato diverse tecniche di conservazione del pesce, mirate a garantire la preservazione delle sue qualità. Il metodo di conservazione più noto è la salatura. L'obiettivo di questo lavoro è descrivere la tecnologia di produzione delle sardine sotto sale pescate in mare aperto ed accertare la composizione chimica e la qualità del prodotto finito. Le analisi hanno interessato campioni di sardine sotto sale confezionate in latte da 5 kg. I pesci sono stati campionati mediante il metodo della scelta casuale (randomizzazione) dall'intero contenuto delle latte. Sottoposti ad esame sensoriale, i pesci campionati si sono tutti dimostrati di qualità soddisfacente. L'ispezione della cavità addominale e dei muscoli non ha evidenziato la presenza di parassiti. È stato misurato un valore ponderale medio dei pesci campionati pari a 22,97 g, per una quantità media di acqua pari al 46,61%, di grassi pari all'8,92% e di proteine pari all'8,40%. Nei campioni esaminati è stata accertata una quantità di sale pari al 22,54%. I risultati ottenuti ci portano a concludere che l'impeccabile qualità delle proprietà sensoriali delle sardine sotto sale dipende dal corretto trattamento del pesce dopo la pesca e dal metodo di lavorazione cui sono sottoposte. Per ottenere questo prodotto autoctono è necessario impiegare materia prima – pesce – di qualità (pesce fresco di primissima qualità), salarlo con sale puro nel rispetto del corretto ordine di salatura, stoccarlo in condizioni adatte e controllarlo con regolarità.

Parole chiave: *salatura, sardine sotto sale, qualità, composizione chimica*

Ractopamine – Growth promoter in meat and meat products

Pleadin[*1], J.*

Review

Abstract

Ractopamine is the substance from the group of β-adrenergic agonists, xenobiotic of new generation with evidenced activity as growth promoter in farm animals. Application of ractopamine in pigs in the final stages of fattening resulted in increased body weight and lean meat, reduced amount of fat, accelerating the metabolism of animals and the speed of their fattening. The pharmacological and toxicological effects of ractopamine in humans are still not fully known, but some data suggest that consumption of meat and meat products derived from animals to which ractopamine was applied can resulted with clinical effects and adverse consequences on human health. Application of ractopamine on farm animals results with accumulation of its residues in the internal organs and pigmented tissues. Although the use of substances with anabolic effect is permitted in many countries of the world, the European Union prohibited the application of substances with anabolic effect on farm animals, and also of ractopamine. Therefore, through the annual plans prescribed by the competent authorities continued the monitoring of residues of these substances, by sampling of various animals biological materials on farms and in slaughterhouses, and using sensitive and selective analytical techniques for their detection, with the aim of carrying out a more effective control of abuse of substances that achieve an anabolic effect.

Key words: *ractopamine, β-adrenergic agonists, growth promoter, farm animals, residues*

Introduction

Modern meat industry is today mainly driven by the requirements and desires of consumers, and not, as it decades ago was the case, by the requirements imposed by the production and processing of meat. Consumers require that meat and meat products are lean or contain the least possible amount of fat. In the same way, legal entities that deal with packaging and processing of meat from suppliers demanded that they delivered carcasses of animals with which treatment can be obtain the highest possible yield with less tranching. In order to meet the expectations of today's market, these circumstances led the livestock industry to begin using β-adrenergic agonists (β-agonists), and their use in the meat industry has divided experts worldwide.

The characteristic structure of these substances is very similar to the catecholamines epinephrine and norepinephrine, which are a natural component of the animal body, and which are in medicine and veterinary medicine particularly used as tocolytic agents as also bronchodilators and cardiotonics in treating a pulmonary disease (Meyer Rinke, 1991; Courtheyn et al., 2002). In addition to the legitimate use, in the past was also evidenced many cases of β-agonists abuse, which sought to improve the composition of animal carcasses in terms of reducing the fat content due to increased muscle mass, thus producers realized greater economic benefits (Peter and Scanes, 1990; Anderson et al., 2009; Moody et al., 2000).

Among ß-agonists, ractopamine is pharmacologically classified as a phenethanolamine that presents xenobiotic of new period evidenced as growth promoting agent (EFSA, 2009). In comparison to some other β-agonists ractopamine is not intended for use in humans for any medical purposes. The more probable route of exposure to ractopamine in humans is through the consumption of meat and meat products from animals which have been fed with this anabolic agent which caused the persistence of residues. If it is given to pigs in the final stages of fattening to aim at the final weight gain of an additional 50 kg, ractopamine increases the amount of lean meat and in carcasses of animals reduces the amount of fat, increasing both speed pig fattening, as well as the speed of their metabolism (Anderson et al. 1989; Merkel et al. 1987; Watkins et al., 1990; Williams et al. 1994; Pleadin et al., 2012).

This response of the body is systematically observed in five major species whose meat was consumed by humans, and wherein the sheep and cattle showed greater response than turkeys, pigs showed greater than broiler chickens (Mersmann, 1998; Moody et al., 2000). Biochemical basis of ractopamine effects lies in increasing the retention of nitrogen and protein synthesis, facilitating the suppression of lipolysis and lipogenesis (Apple et al. 2007; Armstrong et al. 2004a; Carr et al. 2005a; Mills, 2002; Mitchell et al. 1990; Mitchell, 2009).

The metabolic effect of ractopamine is similar in pigs, cattle, laboratory animals and humans. It is a cardiac stimulator and has the effect of restricting blood vessels and quickening the heart. In comparison to other beta-agonists such as salbutamol and clenbuterol that can be used in terapeutic purposes, ractopamine is not as toxic to humans, but long-term misuse of ractopamine may still result in potentially harmful side effects (EFSA, 2009). However, clenbuterol among ß-agonists is known to have a much longer half-life in blood than ractopami-

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ne, thus has a greater potential for bioaccumulation. Clenbuterol is reported to induce unintended side effects on humans, such as increased heart rate, muscular tremors, headache, nausea, fever, and chills and its use in food animals has been prohibited in almost all world countries (Pleadin et al, 2012b).

Although all incidents of poisoning in the past were caused exclusively by clenbuterol toxicity, the European Union has placed ban upon the use of all β-agonists, including ractopamine, thus requiring strict monitoring over these substances. However, ractopamine is approved by the U.S. Food and Drug Administration for use in finishing swine and it is currently approved for use in many countries worldwide. Data pointing that opinions of scientists and prescribed legislation around the world differ significantly in terms of justification for the application of ractopamine on animals for meat production in order to achieve better utilization of food and sensory characteristics of meat, as well as the possible adverse consequences that may result from use of this substance. When it comes to the advisability of ractopamine to increase food utilization and sensory characteristics of the meat used in meat industry, reference data indicate to significant global differences of researchers opinion, significant differences in the applied regulations of different countries of the world, as well as to possibility of harmful effects that may arise from the use of this substance.

Mode of action and effects in animals

Phenethanolamines generally are characterized by a substituted aromatic ring and an ethanolamine side chain, binding to the alpha-adrenergic receptors (α-ARs) and beta-adrenergic receptors (β-ARs) located on the surface of the cell. Biological activity of β-adrenergic agonists is attributed to the six-membered aromatic ring, which may be substituted on the aliphatic nitrogen with hydroxyl groups, halogens, amines, hydroxyl methyl groups, cyano groups, or their different combinations. The chemical substitution, especially with halogens, significantly affects the excretion half-time extension of β_2-adrenergic agonists in mammals as well as their affinity for binding to the receptor (Smith, 1998).

There are three subtypes of β-AR, namely β1, β2 and β3, and their distribution varies between species and tissues, but β-ARs are present in almost all mammalian cells. The β1 subtypes are the most abundant receptor subtype present in pig tissues, accounting 80% in adipose tissue, 72% in heart, 65% in lung, 60% in skeletal muscle and 50% in liver (McNeel and Mersmann, 1999; Badino et al., 2005; Pleadin, 2006).

Coupling of ractopamine with β-AR results in activation of Gs proteins, which in turn stimulate adenyl cyclase and resulted in a cascade of reactions that eventually concluded with the phosphorylation of numerous enzymes and regulatory factors important in metabolic regulation (Moody et al., 2000). The latter may trigger a series of physiological responses that result in increased carcass leanness and decrease fat content (Mersmann, 1998). In adipose tissue, activation of β-ARs results in increased protein kinase A activity leading to activation of hormone-sensitivity lipase with subsequent triglyceride hydrolysis.

In addition, protein kinase A also inhibits lipogenesis due to phosphorylation and inactivation of glucose transport and acetyl-CoA carboxylase and reduced expression of lipogenic genes (Mersmann, 1998) finally resulted with reduced carcass fat (Moody et al., 2000). The majority of evidence points out that β-AR are involved in the growth response but it remains uncertain whether the effect of β-AR on skeletal muscle growth are direct or indirect. Furthermore, β-agonists have little effect on the levels of circulating hormones known to influenced growth and metabolism (Moody et al., 2000). A temporary increase in blood flow to skeletal muscles have been reported, which would increase nutrient availability and probably contribute to the growth response (Mersmann, 1998).

The major effect of β-agonists on muscle fiber is to cause hypertrophy without an increase in DNA, suggesting that protein synthesis and degradation, or both, are affected (Mills, 2002). Ractopamine also increased the size and percent of intermediate fibers, fractional protein synthesis and accretion accompanied with an increased in myofibrillar protein synthesis at expense of sarcoplasmic protein synthesis (Adeola et al., 1992). Findings suggest that ractopamine increase muscle mass by increasing protein synthesis with little effect on protein degradation. Moody et al. (2000) concluded that due to differences in βAR subtype specificity of ractopamine (which has a binding affinity to the receptor subtypes β1) in comparison to β2-substances protein degradation is not affected. Moreover, activation of β2 receptors stimulates protein synthesis and a reduction in protein degradation, whereas activation of β1 only stimulate protein synthesis.

Effect on carcass traits and meat quality

Because of their ability to redirect nutrients from the fat to skeletal muscle, thereby increasing the representation of pure lean meat yield, β-adrenergic receptor agonists is often referred as repartition agents (Ricks et al., 1984). Incorporation of ractopamine to the diet of pigs in the final stage of fattening outcomes with increased carcass weight and dressing percentage with little effect on meat quality (Carr et al., 2005a; Carr et al., 2005b; Apple et al., 2007) and also proven positive effects on yield (Crome et al., 1996; Carr et al., 2005a; Fernández-Dueñas et al., 2008; Kutzler et al., 2011). Response to ractopamine is dependent on dosage, duration, and dietary protein. Lower dosages (≤10 mg/kg) results in mild increments in carcass characteristics whereas higher dosages (>10 mg/kg) optimize these characteristics, generally improving the carcass composition (Watkins et al., 1990; Williams et al. 1994; Armstrong et al., 2004).

Some authors reported that in the pigs fed with ractopamine reduction in fat ranging between 7% and 16% (Herr et al., 2001; Apple et al. 2004a; Carr et al., 2005b), while other authors reported similar fat depth between ractopamine and controls pigs (Crome et al., 1996; Leick et al., 2010; Kuztler et al., 2011). These differences can be explained by differences in administered dose of ractopamine. Watkins et al. (1990) reported that in order to achieve the maximum increase in the share of pure lean meat, ractopamine dose equal or greater than 10 mg/kg is required.

Investigations of ractopamine impact to share of lean meat in the carcasses of animals have reported different results. In the study by Armstrong et al (2004) that compared the differences in the effects of dose and duration of ractopamine administration, it was found that increasing the proportion of pure lean meat in the carcasses of animals could be achieved after 27-day of oral ractopamine administration. On the other hand, several authors stated that between pigs fed with ractopamine and control pigs there was no difference in this respect (Brumm et al., 2004; Rincker et al., 2009; Leick et al., 2010). In order to accurately predict the impact of feeding with ractopamine on carcass leanness, more precise methods should be use, such as dissection (separation of lean and fat tissue) and chemical analysis (Schinckel et al. 2003).

Water holding capacity refers to the ability of meat to bind or retained moisture during storage. Literature data shown that ractopamine feeding had no significant effect on percent of drip loss (Stoller et al., 2003). According to meat color, most of the data was collected by the survey of *longissimus musculus* as main indicator of the pork carcass. Introducing ractopamine in the pigs diet had only a slight effect on L* values (lightness meat) or this effect is completely absent. In contrast, the mild but persistent reduction in a* (redness) and b*(yellowness) has been reported in ractopamine fed pigs with the increase in the proportion of muscle white fibres (Aalhaus et al., 1992; Apple et al. 2004b; Carr et al., 2005a; Carr et al., 2005b). This shift in the type of muscle fibers indicates the reduction of myoglobin, because white fibers have less myoglobin than red and intermediate fibers. Regardless of statistical significance, the reduction of a* and b* values is of little practical importance, since it is possible that consumers will not notice any difference between pork derived from pigs fed with ractopamine and pork for which this is not the case.

Measuring the pH value of meat is one of the simplest and most common measurements in the meat industry conducted to determine the meat quality. According to the results of the study conducted by Boler et al (2010), pH was measured 24 hours after slaughtering of the animals proved to be the most reliable indicator of pork color, its capacity of water retention and softness. Most of the researches have been reported that the final pH-value measured in the loins of the animals fed with ractopamine were equal to control animals (Stoller et al., 2003; Carr et al. 2005b). However, some researches have indicated that the final pH values measured in the loins of pigs fed with ractopamine were higher than those measured in the control animals (Apple et al., 2004b; Rincker et al., 2009).

Sensory analyzes conducted by several studies have shown that the intensity of flavor, juiciness and tenderness of ham from pigs fed with ractopamine were comparable to those obtained from control pigs. Generally, the use of ractopamine in the diet of pigs had only a minor impact on the quality of meat or this effect is completely absent (Uttar et al., 1993; Carr et al., 2005a; Carr et al., 2005b; Rincker et al., 2009; Kutzler et al., 2011).

Toxicological effects

The metabolic pathway of ractopamine hydrochloride in various target biological species (pigs and cattle, laboratory animals and humans) is very similar. The pharmacological and toxicological effects of ractopamine in humans is still not fully known. According to some data, ractopamine 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 obtain clinical effects such as tachycardia and other heart rate increases, headache, muscle spasm, or high arterial blood pressure. Among others are effects on metabolic parameters (glucose, free fatty acids), muscle tremor, effects on behaviour (restlessness, apprehension, anxiety) and effects on bronchial hyper-reactivity (EFSA, 2009).

Investigations of mutagenic potential indicate that the ractopamine is not mutagenic. However, the results of several *in vitro* studies, including tests on chromosomal aberrations on human lymphocytes as biologic material, were positive. Positive test results of genotoxicity were explained with limited evidence to be due to a secondary auto-oxidative mechanism from ractopamine-catechol-producing reactive intermediates. Ractopamine is not considered to be a direct carcinogen (carcinogen of the first order). The induction of benign leiomyomas (tumors of smooth muscle) in mice and rats can possibly be due to a general feature of beta-adrenergic activity of ractopamine.

Dose-dependent changes of heart rate and cardiac output are observed within the first hour after administration of ractopamine and gradually return to baseline values. The systolic blood pressure will also increase in a dose-dependent manner, while the diastolic pressure remains unchanged. Skeletal muscle tremor is the most common adverse effect of beta-agonists, and is more likely to be seen after oral administration than after inhalation. Tremor results from an imbalance between fast- and slow-twitch muscle groups of the extremities, and its severity varies greatly between individuals. No such effects were recorded at the NOEL (No Observable Effect Level) determined in the toxicological studies conducted in laboratory animals given ractopamine or in the study in humans on cardiovascular effects of ractopamine (EFSA, 2009).

Feelings of restlessness, apprehension, and anxiety were reported effects after the use of various beta-agonists, particularly after oral or parenteral treatment. In pilot clinical trials with ractopamine, four patients showed little evidence for central nervous system stimulation. It is unclear whether long-term treatment with this substance results in the development of tolerance to these adverse effects (WHO Food Additives Series 53).

Analytical methods and control materials

As β-agonists are classified into group A - substances having an anabolic effect and unauthorised substances, in the European Union confirmation of the target analyte is necessary according to analytical performance criteria of the Commission Decision 2002/657/EC. As a result of the requirement of analytical criteria on confirmatory methods, mass spectrometry techniques are widely used to identify residues levels of these substances (Shao et al., 2009).

A number of confirmatory and screening methods, in most cases gas chromatography (GC) or liquid chromatography (LC) coupled with mass spectrometry (MS), or screening method ELISA, are available for the analysis of ractopamine in a variety of matrices. For confirmation a minimum of four characteristic ions are required to satisfy the four identification points for the GC-MS or LC-MS techniques and one precursor ion and two daughter ions can provide four identification points for tandem mass spectrometry techniques including triple quadropole mass spectrometry and ion trapping techniques (e.g. LC-MS/MS and GC-MS/MS) (Pleadin et al., 2012a).

There are literature reports on ractopamine determination by use of different techniques (Shelver and Smith, 2003; Thompson et al., 2008; Turberg et al., 1995). However, GC/MS methods require derivation step because of their high polarity and low volatility, which is time-consuming, tedious, laborious and expensive (Shao et al., 2009). Because of its high sensitivity and selectivity, LC-MS/MS is often the method of choice in the analysis of trace levels of polar contaminants (Nielen et al., 2008; Pleadin et al., 2011) and recently many authors demonstrated its superior performance in ractopamine analyses (Blanca et al., 2005; Churchwell et al., 2002; Smith and Shelver, 2002). So far, ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) is one of the most efficient methods (Nielen et al., 2008; Shao et al., 2009) because of the high resolution, rapid separation of UPLC, and the selectivity and sensitivity characteristic for MS-MS detection (Dong et al., 2011; Zheng et al., 2010).

After treatment of various animal species with ractopamine, and application of different treatment conditions, several authors (Smith et al. 1993, Elliott et al., 1998; Qiang et al. 2007; Shelver Smith, 2002, Thompson et al. 2008; Pleadin et al., 2012; Pleadin et al., 2012c) pointed to the accumulation of residues of these type of substances in the internal organs (liver, kidney, adipose tissue, etc..) and pigmented tissues (hair, eye) of animals (Antignac et al., 2002; Blanca et al., 2005; Dickson et al., 2005; Moragues and Igualada, 2009; Nielen et al., 2008; Thompson et al., 2008; Vulić et al., 2012; Pleadin et al., 2013; Pleadin et al. 2013b). It was also demonstrated that in pigs ractopamine was rapidly eliminated from the body, leaving only a negligible amount of residues, wherein the major elimination pathway was conjugation with glucuronic acid and the excretion by urine (Dalidowicz, 1992). Therefore, the urine is typically used as a control material in investigations of its abuse during fattening.

Relevant legislation and control of abuse

With regard that toxicological studies of short-acting β-agonists ractopamine did not give significant results about the harmful effects on human health and animals, when it was applied in the prescribed anabolic dose, ractopamine use in some countries such as USA, Mexico, Brazil and in South Africa is allowed. In the present, two compounds are approved by the Food and Drug Administration (FDA) to use in meat production animals; Zilpaterol (trade name Zilmax®, Intervet division of Schering-Plough Animal Health, Summit, NJ) for beef cattle and Ractopamine hydrochloride (RAC – Elanco Animal Health division of Eli Lili, Greenfield, IN) marketed under the following trade names: Optaflex®, Paylean® and Topmax® for beef, pork and turkey production, respectively. Paylean® is currently labeled for use at a dose of 5 – 10 mg/kg for the last 20.5-40.9 kg of gain prior to slaughter in pigs initially weighing at least 68 kg.

Currently, ractopamine is permitted in 27 countries to be used during the fattening of pigs (Mitchell and Dunnavan, 1998; Anderson et al., 2009) with defined MRL (maximum residue level) as limit in which it may be present in certain animal tissues (meats) (Heitzman, 1993; Qiang et al., 2007). In the USA the safe concentrations for total residues of ractopamine hydrochloride are: 0.25 mg/kg in muscle, 0.75 mg/kg in liver, and 1.5 mg/kg in kidney and fat. The acceptable daily intake (ADI) for total residues of ractopamine is 1.25 micrograms ractopamine hydrochloride per kilogram of body weight per day. The human safety derived for meat products as food of animals that were fed with ractopamine has been documented by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2004).

However, following Council Directive 96/22/EC, placing of β-agonists on the EU market for use in growth promotion on farm animals intended for human consumption is forbidden (without exception). In the Republic of Croatia, in force is Directive on the prohibition of the use of certain substances having a hormonal and thyreostatic action and β-agonists in animal husbandry (NN 51/2013). The Directives prohibits the importation from third countries of farm animals, or their meat, to which β-agonists have been administered (except for the above described therapeutic uses for clenbuterol) and irrespective of any guarantee that the meat is free from residues. Among β-agonists only clenbuterol is used for therapeutic treatment, under direct veterinary supervision, in calving cows, foaling horses and pets. It can be used only in therapeutic purposes for achieving broncholitic and tocolitic action in bovine and equine animals at doses of 10-20 μg twice daily (EMEA, 2000) which is 5-10 times lower than anabolic dose (Smith, 2000; Pleadin et al., 2011).

However, clenbuterol is pointed out as the most important and most effective member of β-agonists group, in terms of the anabolic application and consequent many intoxications of people in the past because of its misuse (Smith and Paulson 1997; Brambilla et al., 2000; Dunshea et al., 2005; Pleadin et al., 2009; Pleadin et al., 2011). Due to the possible use of clenbuterol for therapeutic purposes, in order to establish control over the possible abuse as anabolic, European legislation set 0.5 μg/kg as MRL for clenbuterol, referring thereby exclusively on the liver of cattle and equine. Ractopamine is not used for the stated purposes, only as a substance with anabolic effect, in order to achieve higher yields and profits in the livestock and meat industry.

Conclusion

The fact that the application of β-agonists resulted with higher efficiency of fattening and promotes the growth of animals and a substantial improvement in the redistribution of muscle in relation to fat tissue, has led to a series of abuse of these substances in the process of farm animals breeding. Application of ractopamine, and other substances from this group, resulting with accumulation of residues in various tissues of animals, also in meat and meat products, whose consumption at the same time can cause intoxication in humans. Therefore, in the EU, and also in Croatia, surveillance programs of substances with anabolic effect have been performed continuously, within the annual plans established by state bodies. The supervision should be conducted during the fattening of animals on farms and in slaughterhouses, by sampling of various biological materials and the application of sensitive and selective analytical techniques in their detection, with the aim of systematic control over the use of ractopamine and other substances which result with the anabolic effect in farm animals.

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Consistent implementation of the residue monitoring plan for consumer health protection

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Case report

Summary

In order to protect consumer health and to create conditions for the trade of live animals and animal products with the European Union, and with third countries wishing to trade these kind of products with the European Union, there is legal obligation to monitor and control plans. Residues are pharmacologically active substances, which may remain in live animals and animal products, and which may be suspected or proven to be harmful to human health. The implementation of the monitoring plan for residues ensures a systematic method for revealing the possible presence of illegal drugs or certain substances above permissible limits in live animals and animal products. Once residues are detected in samples submitted for laboratory testing, the competent inspection authorities are required to implement all the necessary follow-up measures in order to determine the causes and origins of illegal drugs, or the reasons that led to exceeding the maximum allowable amount in foods of animal origin intended for human consumption. This paper describes a follow-up procedure conducted after the detection of coccidiostats in table eggs from farm hens.

Keywords: *Residues, plan, follow-up, coccidiostats*

Introduction

Today's lifestyle and intensive animal production have resulted in human exposure to a variety of adverse effects, such as residues of veterinary drugs, hormones, contaminants and other toxic substances. People are able, as individuals, to reduce these effects to a certain extent, though in most cases, it is necessary to provide an integral system that combines multi-disciplinary science, to ensure protection of the individual. This study gives a practical example describing a systematic approach to protecting the consumer health, i.e. protecting the entire human population from the harmful effects of residues of veterinary medicines (coccidiostats). These may remain in live animals and animal products and enter the human body through the food chain, thus jeopardizing health. This example outlines an effective way in which the profession acted to overcome the shortcomings of individual entities. It represents years of applying this systematic approach to passing legislation, its implementation, proper training, technical equipment and professional training of laboratory staff, and the permanent commitment of enthusiastic individuals. This example will show how the smallest error in the manufacturing process can result in serious consequences for human health and can incur serious financial costs. It also shows that the right implementation plan for residue monitoring can prevent such consequences or at least reduced them to an acceptable level. In order to protect the personal and business interests of the companies involved, the personal data of individuals, details of food business operators, or year of events will not be divulged.

Residue Monitoring Plan

In addition to the primary objective of consumer protection, the systemic control of substances is intended to ensure the conditions for free trade in live animals and animal products. To this end, the European Union passed Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products, which clearly lays down the necessary measures to be implemented for monitoring substances and groups of residues listed by importance of this segment for the protection of human health (EC, 1996). This includes monitoring the production process of animals and primary products of animal origin, with the aim of detecting the presence of residues of certain substances in live animals, their excrement and body fluids, tissues, animal products, animal feed and drinking water. The Directive also regulates the coordination of work of inspectors implementing the regulation on the ground within their national territory and the central competent authority. In the case the maximum allowable quantity of residues of a certain substance is exceeded, the competent authority is required to conduct an investigation on the farm of origin, and to determine the cause of exceeding the allowable limit. If the presence of prohibited substances or products is detected by unauthorized persons, the detected substance or products is placed under the control of authorized persons until the competent authority implements the adequate measures. This Directive also stipulates the measures to be fulfilled when imported from third countries, and the manner of sampling. All third countries wishing to trade in live animals and animal products with EU Member States are obliged to

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