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Review

Current research, regulation, risk, analytical methods and monitoring results for nicarbazin in chicken meat: A perspective review

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ABSTRACT

This review presents up-to-date information about current research on nicarbazin, one of the most used anticoccidials in poultry production. The focus is to elucidate regulation concerning nicarbazin, limits for its residues in food, how maximum residue limits in different countries are calculated regarding edible chicken tissues and the possible implications in human health. Analytical methods to extract and quantify this residue, expressed as dinitrocarbanilide (DNC) are presented and discussed, including qualitative screening and quantitative/confirmatory analytical methods. Monitoring results and occurrence of DNC residues in chicken meat are discussed. Additionally, the causes of eventual chicken meat contamination and possible solutions to reduce or eliminate DNC residue in tissues are also presented. The paper concludes with perspectives, the current state of DNC residue analysis and suggestions for future research, especially considering the gap in the study of residue recycling effect due to continuous chicken litter use.

1. Introduction

Chicken meat has a great nutritional value and is recognized as a healthy and low-cost source of animal protein, which makes it accessible to low-income families worldwide. While the United States is positioned as the world's leader in poultry production, Brazil is the leading broiler meat exporter in international trade (ABPA, 2016; USERS, 2016). One important issue in this meat market is related to food safety, especially concerning chemical residues in the final products. This relates to nicarbazin, a product of chemical synthesis widely used in commercial intensive broiler farming to control the coccidiosis.

Coccidiosis refers to a disease caused by protozoa of the *Eimeria* genus promoting a wide range of injuries in poultry intestines. Parasites compromise the nutrient absorption and feed conversion, affecting poultry weight gain or even causing mortality, as a consequence of intestine damage (Blake & Tomley, 2014; Chapman, 2014). The extent of losses caused by *Eimeria* spp. in poultry flocks was relieved with the advent of anticoccidials in the 1950s. Nicarbazin, one of the first anticoccidials developed, is still being used successfully in prophylactic programs. However, considering recent concerns with undesirable levels of residues of this feed additive in chicken products intended for human consumption, the aim of this article is to contribute to the understanding about safety related to nicarbazin residues (expressed as DNC) in chicken tissues, considering important common questions of any consumer: What would be the possible effect of DNC residues on humans after consumption of contaminated food? How are the acceptable residue levels calculated in major chicken producing and consuming countries, notably, the US, Brazil, and the European Union? How is nicarbazin used in poultry production, and what are safety measures concerning the residue in the final products? Which analytical methods have been employed to quantify DNC residues in foods? Which strategies/researches regarding

Abbreviations: ADI, acceptable daily intake; bw, body weight; CC_α, limit of decision; CC_β, detection capability; DNC, 4-4-dinitrocarbanilide; EFSA, European Food Safety Authority; ELISA, enzyme-linked immunosorbent assays; ESI, electrospray ionization; FCIAS, flow cytometry immunoassays; FPIAs, fluorescence polarization immunoassays; HDP, 2-hydroxy-4,6-dimethyl-pyrimidine; HPLC, high performance liquid chromatography; IC_{50}, inhibitory concentration 50%; LC-MS, liquid chromatography coupled to mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LFDs, lateral flow devices; LOD, limit of detection; LOQ, limit of quantification; LSE, liquid-solid extraction; MAPA, Ministry of Agriculture, Livestock and Food Supply; MRL, maximum residue level; MU, measurement uncertainty; NRP, National Residue Program; NOEL, no observed effect level; PLE, pressurized liquid extraction; PNCRC, National Plan of Residues and Contaminants Control; QuEChERS, quick, easy, cheap, effective, rugged and safe; qToF, quadrupole time-of-flight; SPE, solid phase extraction; SPR, surface plasmon resonance; UHPLC, ultra high performance liquid chromatography

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the use of nicarbazin can be applied to improve food safety? What does the data show about DNC residue in edible chicken tissues?

2. Nicarbazin

The chemical substances that act specifically in preventing and combating coccidiosis are known as anticoccidials, which are classified according to the mode of action into coccidiostat (inhibit *Eimeria* growth) and coccidicide (promote life cycle interruption and parasite destruction). The use of anticoccidials is necessary to maintain health, animal welfare and suitable feed conversion, consequently avoiding broiler poor growth and mortality. Based on their origin, anticoccidials are classified as synthetic (nicarbazin, diclazuril, robenidine, clopidol, and halofuginone) or ionophores (narasin, lasalocid, monensin, maduramicin, senduramicin, and salinomycin). While synthetic anticoccidials are manufactured through chemical reactions, ionophores are produced by different bacteria of the Streptomycetaceae family (Clarke et al., 2014). These products are commercially available and may be employed at different stages of the poultry life cycle, either alone or combined (Clarke et al., 2014; Spínosa, Palermo-Neto, & Górniak, 2014). Nicarbazin, an anticoccidial synthesized in 1955, is still one of the most efficient products used in broiler chicken feed by the largest broiler meat producers and exporters worldwide.

It consists of an equimolar complex (Fig. 1) which undergoes fast in vivo dissociation into two substances: 4-4-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethyl-pyrimidine (HDP). The active component is DNC (solubility in water is lower than 0.02 mg/L) while HDP (hydrophilic) increases DNC absorption. The effectiveness of the complex is higher than DNC used alone. Nicarbazin presents an effective anticoccidial activity, acting as either coccidiostat or coccidicide (Berchieri Júnior, Silva, Fábio, Sesti, & Zuanaze, 2009; EFSA, 2003), promoting adequate coccidiosis control. No resistance incidences that could compromise coccidiosis control have been reported so far (EFSA, 2003; Rogers et al., 1983; Spínosa et al., 2014). Thus, this compound is still widely used in poultry industry (Chapman, 2014).

Regarding the use of anticoccidials, the main safety measures to assure food safety in poultry production are to follow the regulated dosages and recommended withdrawal periods. In Brazil, the use of nicarbazin in broiler feed is regulated by the Ministry of Agriculture, Livestock and Food Supply (MAPA). A maximum concentration of 125 mg/kg nicarbazin of this product is allowed in feed, with a withdrawal period of 10 days before slaughter (MAPA, 2015c; Poli-Nutri, 2016). In the European Union, the maximum amount of nicarbazin in feed is the same as Brazil, but the minimum withdrawal period is one day (EC, 2010a). Meanwhile, in the USA, the amount of nicarbazin authorized in feed is up to 181.6 mg/kg, with a withdrawal period of 4–5 days (GPO, 2012).

When nicarbazin is co-administered with other anticoccidials, there are specific rules regarding to dosage and withdrawal period in different countries. Brazil allows nicarbazin association with maduramicin (40:3.75), monensin (40:40 to 50:50), narasin (40:40 to 50:50) or senduramicin (40:15 to 48:18) in the mentioned concentrations (mg/kg) (Biofarma, 2016; Elanco, 2016; Huvepharma, 2016; Phibro, 2016). In the EU, only narasin is allowed to be associated in a dosage of 40:40 to 50:50 mg/kg (EC, 2010a, 2010b), as it is in the USA at 27:27 to 45:45 mg/kg (GPO, 2012).

2.1. Toxicity

Toxic reactions generated by anticoccidials may occur in different species of animals, including broilers, depending on the administration levels (U.S. National Library of Medicine, 2013). Nicarbazin levels above 125 mg/kg may lead to poultry poisoning, mainly affecting the thermoregulatory mechanisms. Therefore, the use of nicarbazin is generally limited to the initial growing phase, since it may increase heat stress effects in later periods, leading to mortality (Spínosa et al., 2014).

A no observed effect level (NOEL) is defined as the higher level of exposure of an organism, at which no statistically significant adverse effect occurs in the exposed population (EFSA, 2003, 2010a). The concentration of 240 mg/kg_{bw} of nicarbazin a day was established by Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1999), while EFSA (2010a) proposed 154 and 51 mg/kg_{bw} of DNC and HDP, respectively, based on chronic toxicity studies in rats and dogs. The observed effects were weight loss and changes in organs such as kidney and liver (EFSA, 2010a).

Developmental toxicity studies in rats showed maternal weight loss and fetus abnormalities at the highest nicarbazin concentration. Therefore, a NOEL of 200 mg/kg_{bw} a day for maternal and fetal toxicity was established (JECFA, 1999).

Regarding mutagenicity, nicarbazin presented genotoxicity for *Salmonella* spp. However, in vivo tests with mammals did not confirm this effect and nicarbazin was considered genotoxic risk-free. An impurity resulting from DNC synthesis is *p*-nitroaniline, which should be below 0.1% in nicarbazin, based on genotoxicity and carcinogenicity assessments (EC, 2010a; EFSA, 2010a).

The European Food Safety Authority (EFSA) claims that nicarbazin has low acute toxicity (EFSA, 2010a). In fact, in humans acute toxicity has not been reported so far, but there is concern over chronic toxicity when people are subjected to low levels of the anticoccidial during long-term exposure. Many countries are implementing surveillance programs to monitor and prevent unacceptable contamination with anticoccidial residues in animal products intended for human consumption (Clarke et al., 2014).

2.2. Acceptable daily intake

Toxicological information has been serving as a basis for the establishment of the acceptable daily intake (ADI) for humans. ADI is derived from NOEL with the application of an uncertainty factor. Then, the maximum residue levels (MRLs) have been established for foods of animal origin, in order to follow ADI and to avoid risks to consumer (Dorne et al., 2013).

Nicarbazin ADI was established based on a NOEL value of 200 mg/kg_{bw}/day, obtained from the development toxicity evaluation in rats and from a safety factor of 500 (high due to limitations of the available data). Therefore, a calculated nicarbazin ADI of 0.4 mg/kg_{bw} has been established by *Codex Alimentarius* (FAO/WHO, 2015; JECFA, 1999), as presented in Table 1. According to this report, it has been assumed that nicarbazin at 0.4 mg/kg_{bw} corresponds to 24 mg of DNC for a 60 kg person, although NOEL was obtained in terms of nicarbazin, not DNC. Alternatively, European Union considers DNC instead of nicarbazin and has adopted 0.77 mg DNC/kg_{bw} as ADI (corresponds to 46 mg of DNC,

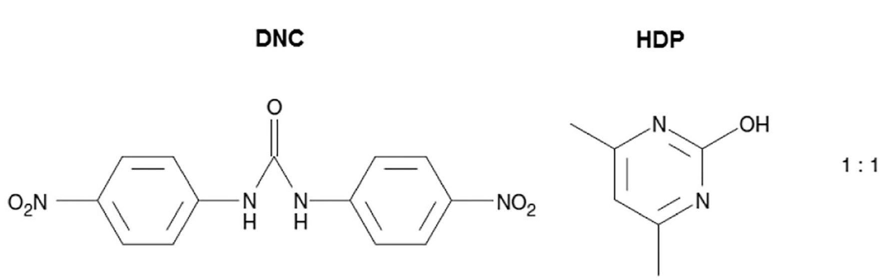

Fig. 1. Structural formula of the nicarbazin equimolar complex (DNC plus HDP).
Source: Tarbin et al. (2005).