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Contemporary Issues in Toxicology

Nitrite in feed: From Animal health to human health

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ABSTRACT

Nitrite is widely consumed from the diet by animals and humans. However the largest contribution to exposure results from the in vivo conversion of exogenously derived nitrate to nitrite. Because of its potential to cause to methaemoglobin (MetHb) formation at excessive levels of intake, nitrite is regulated in feed and water as an undesirable substance. Forages and contaminated water have been shown to contain high levels of nitrate and represent the largest contributor to nitrite exposure for food-producing animals. Interspecies differences in sensitivity to nitrite intoxication principally result from physiological and anatomical differences in nitrite handling. In the case of livestock both pigs and cattle are relatively susceptible. With pigs this is due to a combination of low levels of bacterial nitrite reductase and hence potential to reduce nitrite to ammonia as well as reduced capacity to detoxify MetHb back to haemoglobin (Hb) due to intrinsically low levels of MetHb reductase. In cattle the sensitivity is due to the potential for high dietary intake and high levels of rumen conversion of nitrate to nitrite, and an adaptable gut flora which at normal loadings shunts nitrite to ammonia for biosynthesis. However when this escape mechanism gets overloaded, nitrite builds up and can enter the blood stream resulting in methemoglobinemia. Looking at livestock case histories reported in the literature no-observed-effect levels of 3.3 mg/kg body weight (b.w.) per day for nitrite in pigs and cattle were estimated and related to the total daily nitrite intake that would result from complete feed at the EU maximum permissible level. This resulted in margins of safety of 9-fold and 5-fold for pigs and cattle, respectively. Recognising that the bulkiness of animal feed limits their consumption, these margins in conjunction with good agricultural practise were considered satisfactory for the protection of livestock health. A human health risk assessment was also carried out taking into account all direct and indirect sources of nitrite from the human diet, including carry-over of nitrite in animal-based products such as milk, eggs and meat products. Human exposure was then compared with the acceptable daily intake (ADI) for nitrite of 0-0.07 mg/kg b.w. per day. Overall, the low levels of nitrite in fresh animal products represented only 2.9% of the total daily dietary exposure and thus were not considered to raise concerns for human health. It is concluded that the potential health risk to animals from the consumption of feed or to man from eating fresh animal products containing nitrite, is very low.

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Introduction

Nitrite, the anion of inorganic nitrite salts such as sodium nitrite, is formed naturally by the nitrogen cycle during the process of nitrogen fixation and is subsequently converted to nitrate, a major plant nutrient and constituent. Livestock feeding-stuffs contain both nitrite and nitrate and the latter is converted to nitrite and other metabolites (nitric oxide (NO) and N-nitroso compounds) either in the saliva of most monogastrics or in the fore-stomach/rumen of ruminants due to microbial activity. Adverse health effects in livestock and humans, resulting from acute and sub-acute exposure to excessive nitrite are typically due to the formation of MetHb in the blood. This can lead to cyanosis and at very high levels, death. The consequence of chronic exposure to nitrite is controversial with equivocal evidence of gastric carcinogenicity in female mice (Maekawa et al., 1987; NTP, 2001) but no clear evidence for direct carcinogenic potential in man. In order to protect animal and human health, the European Union, directive, 2002/32/EC on undesirable substances in animal feed, restricts the maximum content of nitrite in complete feeding-stuff (with a moisture content of 12%) for livestock excluding birds and aquarium fish to 15 mg/kg, and the maximum content of fish meal to 60 mg/kg. The current review highlights, the recent risk assessment performed by the scientific panel on contaminants in the food chain (CONTAM Panel) of the European Food Safety Authority (EFSA) regarding the impact of nitrite in animal feed for livestock health and human health following the consumption of animal products such as milk, meat and eggs, (EFSA, 2009). Particular focus is given to toxicokinetic and toxicological aspects in food producing animals, laboratory animals and humans to help explain the potential health impacts of dietary nitrite. Finally, a risk characterisation comparing exposure scenarios in animals and humans and safe levels of exposure concludes the review.

Hazard identification and characterisation

Toxicokinetics of nitrite and nitrate

The toxicokinetics of nitrate have been reviewed elsewhere in detail for non-ruminant and ruminant livestock species, laboratory and companion animals and humans (EFSA, 2008, 2009). Interspecies differences in toxicokinetics for nitrite and nitrate provide a valuable physiological basis to identify potentially susceptible species and populations to the toxicity of both anions. Such interspecies differences are illustrated in Fig. 1 for pigs, cows and humans.

The oral rate of absorption of nitrite and nitrate is low in non-ruminants (pigs) and ruminants since minor amounts (10–20%) pass from the stomach and the rumen respectively to the blood stream as nitrite (EFSA, 2009). In contrast, oral absorption of both nitrite and nitrate is high in rodents and humans (90–95%) (Kortboyer et al., 1997). After absorption, nitrite is rapidly distributed in the plasma and binds to erythrocytes. Interspecies differences in volume of distribution of nitrite have been documented with 1624, 278 and 192 ml/kg body weight (b.w.) in the dog, sheep and pony, respectively, after intravenous administration of 20 mg/kg sodium nitrite b.w. (Schneider and Yeary, 1975). In contrast to nitrite, interspecies differences in the volume of of distribution for nitrate are low ranging between 210 and 330 ml/kg b.w. in humans, ponies, sheep and goats (Schneider and Yeary, 1975; Schultz et al., 1985; Lewicki et al., 1998; EFSA, 2008).

After binding to the erythrocyte s membrane, nitrite is reduced to NO (by xanthine oxidase and NO synthase) which has a wide range of physiological functions in health and disease as a second messenger (Lundberg et al., 2008; Webb et al., 2008). In monogastrics (humans, dogs and mini-pigs), 5–7% of the absorbed nitrate is concentrated from the plasma to the saliva through an entero-salivary recirculation pathway and reduced to nitrite by the nitrite reductase from commensal bacteria present on the back tongue. Approximately 20% of the salivary nitrite is then swallowed into the stomach where it is reduced to NO, oxidised to nitrate in the plasma and re-circulated through the entero-salivary circulation (EFSA, 2008). In contrast, under the acidic conditions of most monogatrics stomachs (pH<3.5) nitrate is metabolised to nitrous acid which in turn spontaneously decomposes to nitrogen oxides including NO (Wright and Davison, 1964; Mirvish, 1975). Endogenous production of NO occurs in the urea cycle using L-arginine as a substrate and NO synthase. However, it has been estimated that exogenous intake of nitrite/nitrate leads to NO levels, in the upper intestine, up to 10,000 times higher than that from endogenous production (McKnight et al., 1997).

Conditions of excessive plasma nitrate result in reduction to nitrite; nitrite reacts with Hb to produce MetHb which can be reduced back to Hb via MetHb reductase (Fig. 1). Physiological levels of MetHb in the human blood range between 1 and 3%, and reduced oxygen transport has been noted clinically when MetHb concentrations are above 10% (Walker, 1990; FAO/WHO, 2003a,b). Cyanosis and hypoxia occurs above 20%, and levels above 50% can be life threatening (Mensinga et al., 2003). Infants under 3 months of age are more susceptible to MetHb due a 40–50% lower MetHb reductase levels compared with adults. Moreover, in foetuses and neonates, Hb has a higher affinity for oxygen and hence forms MetHb more readily than adults (WHO, 1997). Finally, because of a relatively high gastric pH, infants have an increased likelihood of intestinal infections where pathogenic bacteria can rapidly reduce nitrate to nitrite (Savino et al., 2006).

Interspecies variability in MetHb reductase activity has also been estimated, as a percentage of human activity and partially accounts for differences in sensitivity to MetHb between species. Pigs have been shown to lower MetHb reductase (27%) compared with horses (63%), cattle, cats and goats (90%), dogs (114%), sheep (150%) and rabbit (452%). Such low MetHb reductase in pigs together with relatively low nitrite reductase levels in the saliva provide a metabolic rationale for their physiological sensitivity to nitrite toxicity. Intra-species differences in MetHb reductase activity have also been shown to be associated with congenital defects as well as age-related differences in reductase expression between neonates and older animals (Harvey, 2006).

In contrast to pigs, the rumen and enlarged caecum of cows (in addition to their relatively high pH (>5)) are especially well suited for nitrate reductase activity. This results in only10–20% of nitrite absorption into the blood stream as the bulk is metabolised by rapidly adaptable gut flora to ammonia for onward synthesis into amino acids and protein or eliminated with other gases during eructation (Lewis,