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Quantification and feed to food transfer of total and inorganic arsenic from a commercial seaweed feed

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ABSTRACT

Seaweed has a long-associated history of use as a supplemented livestock feed, providing nutrients and vitamins essential to maintaining animal health. Some species of seaweed, particularly the fucoids, are well-known accumulators of the metalloid arsenic (As). Arsenic toxicity to humans is well established even at low exposure levels and is considered a class 1 human carcinogen. As mankind's appetite for livestock produce continues to grow unabated, there is a concern that consumption of livestock produce reared on a diet supplemented with seaweed animal feed (SAF) may pose a threat to the human population due to potentially high levels of As present in seaweed. To address this concern and provide end users, including industry, consumers, policymakers and regulators with information on the exposure associated with As in commercial seaweed animal feed, the estimated daily intake (EDI) of As was calculated to evaluate potential human exposure levels. Using As data from a commercially available seaweed meal over a five-year period (2012–2017) a population exposure assessment was carried out. A Monte Carlo simulation model was developed to characterise the feed to food transfer of As from animal feed to animal produce such as beef, milk, chicken, and eggs. The model examined initial levels in seaweed, inclusion rate in animal feed, animal feeding rates and potential transfer to food produced from a supplemented diet of SAF. The analysis of seaweed animal feed showed that inorganic As was a small fraction of the total As found in seaweed meal (80:1). Statistical analysis found significant differences in the concentration of As in seaweed animal feed depending on the grain size ($p < 0.001$), with higher As concentrations in smaller sized grain fractions. Due to several detoxification steps and subsequent rapid excretion from the bodies of livestock, a very low carryover rate of As compounds from seaweed animal feed into livestock produce was observed. The EDI calculated in this study for the livestock produce evaluated at the 95th confidence interval was < 0.01% of suggested safe levels of inorganic As intake. The threat to the general population as a result of consumption of livestock products reared on a diet consisting of SAF is found to be negligible.

1. Introduction

Consumption of livestock and livestock produce contribute 12.9% of global calories and 27.9% of global protein through the provision of meat, milk, eggs, and offal (FAO, 2011). In response to population growth and subsequent food demand, global livestock production is forecasted to increase by 60–70% by 2050 (UN, 2007; Makkar et al., 2015). It is important, therefore, that care is taken in the provision of safe animal feed. The global animal feed market is currently valued at $460 billion and equates to a total annual global production of 980 million tonnes, with 439 million and 184 million tonnes produced for poultry and cattle, respectively (Alltech, 2015). The most recent surveys indicate that the global production of animal feeds has surpassed 1 billion tonnes (Alltech, 2016).

The global seaweed animal feed (SAF) market is worth $11.34 billion annually and accounted for roughly 2.5% of the global animal feeds market in 2016. Conservative estimates of the current value of the seaweed industry are US $10.1–16.1 billion, with projections of market growth to reach US $17.6 billion by 2021 (White and Wilson, 2015; Marketsandmarkets, 2016). Seaweed animal feed can play an important role in the diet of livestock as it is rich in amino acids, trace elements, antioxidants, and vitamins, while also assisting in nutrient absorption

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(Rey-Crespo et al., 2014). The brown seaweed *Ascophyllum nodosum* (Linnaeus) Le Jolis is the main algal species used for the production of livestock feed in Europe and North America and is exported globally to markets in Asia, Australia and South America (Makkar et al., 2015; Mac Monagail et al., 2017).

The benefits of seaweed inclusion in the animal diet are well documented (Brown et al., 2014). However, the production of seaweeds suitable for animal feeds are not without issues; for instance, the uptake of metals from the surrounding water is a phenomenon characteristic of seaweeds (Utomo et al., 2016) and *A. nodosum* has been widely used as a biomonitor of metal contamination in the marine environment (Morrison et al., 2008). Brown seaweeds, in particular, have a tremendous capacity to accumulate As (As being enriched in *Laminaria* species by a factor 200–500 compared with As in terrestrial plant material) (Morrison et al., 2008; Ratcliff et al., 2016). Weathering of As-containing rocks liberates inorganic forms of As, namely arsenic trioxide, arsenite, and arsenate, and is considered a major natural source of As distribution in the ocean (Ryan et al., 2015). The most common inorganic arsenic (As_{Inorg}) species in seawater is arsenate, with typical levels of 1.5 $\mu g\,L^{-1}$ found (range: 1–2 $\mu g\,L^{-1}$) (Smedley and Kinniburgh, 2002).

Total arsenic (As_{Tot}) is the most commonly recorded As value in the scientific literature. However, having little toxicological significance due to its ill-defined toxicity, it is difficult to draw conclusions from an As_{Tot} value (Petursdottir et al., 2015). Speciation information provides defined information on the potential risks associated with consumption of certain products. In isolation, As_{Tot} is not an adequate tool to use in the exposure assessment of As and one cannot infer adequate information on As toxicity and bioavailability as a result. In seaweeds, over 100 major As species, including organobetaine, organochlorine and a number of dimethylarsinyl riboside derivatives of organosugars have been identified (Andrewes et al., 2004; Francesconi, 2010; Navas-Acien et al., 2011). Compounds of As vary in toxicity with inorganic arsenic (As_{Inorg}) considered more toxic than organic species (As_{Org}) (Brandon et al., 2014). Organoarsenicals present in seaweeds, and other marine organisms are loosely considered nontoxic (Niegel and Matysik, 2010). The metabolism of arsenosugars in humans is, however, inherently dependent upon the metabolism of the individual (Feldmann and Krupp, 2011) and caution should be exercised when considering the toxicity of arsenosugars.

It was important to determine the potential human exposure to As as a result of consuming livestock meat, milk, and eggs as “any risk assessment of undesirable substances in feeds needs to consider the occurrence and exposure for consumers of these animal-derived products” (Dorne and Fink-Gremmels, 2012). Humans are routinely exposed to As in the environment via consumption of food and drinking water (Hughes et al., 2011; Morrison et al., 2016; Davis et al., 2017; McGrory et al., 2017; Monrad et al., 2017). Debate and ambiguity, however, surrounds the determination of acceptable exposure levels for various As compounds (Gentry et al., 2014). Inorganic arsenic is categorised as a Group A human carcinogen by the United States Environmental Protection Agency (USEPA), and a Class 1 carcinogen by the International Agency for Research on Cancer (IARC) (Straif et al., 2009). The strong affinity for As uptake, coupled with the perennial growth of fucoids may result in its accumulation at elevated concentrations proving potentially hazardous to human health (Hwang et al., 2010). Limits on As_{Inorg} in seaweeds for human consumption vary globally. In France, the maximum allowable level of As_{Inorg} in food is $< 3.0\,\mu g\,g^{-1}$, while in Australia and New Zealand a limit of 1 $\mu g\,g^{-1}$ is in place (Mabeau and Fleurence, 1993; ANZFA, 2013). In animal feed, the maximum allowable concentration under European regulations is set at 40 $\mu g\,g^{-1}$ for As_{Tot} and 2 $\mu g\,g^{-1}$ for As_{Inorg} (Commission Regulation (EU) 2015/186) (EU, 2015). Historical incidences of mycotoxin (*Fusarium*) contamination of animal feeds (Coffey et al., 2009) has drawn worldwide attention to the animal feeds industry and has resulted in increased scrutiny (Binder et al., 2007; Antonissen et al., 2014; Zachariasova et al., 2014). Although meat (beef and chicken), milk and eggs are widely consumed, to the best of the authors' knowledge no human exposure assessment or estimation on As in seaweed animal feed has been undertaken. Therefore, this study aims to improve our understanding of the potential human exposure to As associated with the consumption of livestock (livestock products) raised on *A. nodosum* animal feed. The exposure to As by the studied population from consumption of bovine and poultry produce fed SAF was estimated. A Quantitative Exposure Assessment (QEA) methodology was used to assess the probability and severity of potential As transfer to humans. This exposure assessment will provide end users including industry, consumers, policymakers, and regulators with information on the exposure levels associated with As in commercial seaweed animal feed and evaluate the provision of safe animal feed, addressing seaweed quality issues.

2. Materials and methods

2.1. Seaweed animal feed (SAF)

For the purpose of this study, any reference made to beef, poultry, milk or eggs refers to those commodities, which have been produced from a diet consisting of SAF. Fig. 1 highlights the basic transport route of As into humans from SAF.

The data used in this study originated from the monthly monitoring and testing of total and inorganic As in a commercial, internationally available SAF (*A. nodosum*) between January 2012 and February 2017. During this period, total As was determined in 62 feed batches, and inorganic As in 60 batches (As_{Tot} $n = 62$; As_{Inorg} $n = 60$) in two different grain size fractions of the SAF (Small Grain (SG); 850–250 μg) and Large Grain (LG); 1940–850 μg).

2.2. Study area and sample preparation

The location from which *A. nodosum* was harvested for the production of SAF extends from 54° 20′ 58.8732″ N, 9° 48′ 2.592″ W to 53° 11′ 50.3772″ N, 8° 59′ 25.7244″ W over a 1000 km stretch of the Atlantic coastline of Ireland. The intertidal lithologies from these harvesting areas comprise igneous, sedimentary and metamorphic bedrock types (Hepworth Holland and Sanders, 2009; Morrison et al., 2009; Guiry and Morrison, 2013). The study area contains a comparatively low human population density with relatively little heavy industry and subsequent low inputs of wastes into the coastal water (Morrison et al., 2008; Morrison et al., 2017; Wilkes et al., 2017).

Harvested *A. nodosum* is dried before being industrially milled via sieving through multiple screens (ranging from < 250 to 1940 μm) where it is processed into animal feed and exported worldwide.

2.3. Determination of total and inorganic arsenic

On a monthly basis between 2012 and 2017, dry feed samples (~0.5 kg) of LG-SAF and SG-SAF were collected at random positions form three bags of SAF product from a commercial producer in Ireland. All the samples were analysed in the GAFTA (The Grain and Feed Trade Association) approved laboratory (TLR, Netherlands) for the determination of organic and inorganic As fulfilling the requirements of the standard NEN-EN-ISO/IEC 17025:2005. A test portion of 0.3 g of dry feed sample was treated with diluted nitric acid (CARLO ERBA, RS-Superpure for trace analysis, Cornaredo, Italy) and hydrogen peroxide (TraceSELECT® Ultra Sigma-Aldrich, USA) in a heated water bath. Hereby, the As species are extracted into solution and As(III) is oxidized to As(V). The inorganic As is selectively separated from other As compounds using anion exchange high-performance liquid chromatography (HPLC) (Thermo Scientific Dionex UltiMate3000) coupled online to the element-specific detector inductively coupled plasma - mass spectrometry (ICP-MS) (Thermo Scientific X Series II) for the

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