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Dietary rice bran supplementation prevents *Salmonella* colonization differentially across varieties and by priming intestinal immunity

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ABSTRACT

The global burden of enteric dysfunction and diarrhoeal disease remains a formidable problem that requires novel interventions. This study investigated the immune-modulatory capacity of bran across rice varieties with phytochemical differences. 129SvEvTac mice were fed a 10% rice bran or control diet followed by infection with *Salmonella enterica*. Faecal shedding titres were quantified and flow cytometry was used to investigate intestinal immunity. The largest protection against *Salmonella* colonization was observed with IAC600 variety. Reduced faecal shedding correlated with increased levels of boron, soluble fibre, vitamin E isomers, and fatty acids. IAC600 and Red Wells rice bran modulated small intestinal neutrophils, macrophages, interdigitating dendritic cells, $CD8^{+}$, γδ, and regulatory T cells, as well as $CD8^{+}$ and γδ T cells in the mesenteric lymph nodes. Rice bran is a promising functional food and merits evaluation for the prevention of *Salmonella* colonization and regulation of intestinal immunity in people.

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1. Introduction

Enteric disease remains a major global health problem, with diarrhoeal disease remaining a top killer of children in developing nations (Levine, Kotloff, Nataro, & Muhsen, 2012). *Salmonella* spp. are one of a diverse set of enteric pathogens that contribute to diarrhoeal disease in both developed and developing regions of the world with similar incidence rates of 240 and 540 per 100,000 in developed nations of W. Europe and N. America and 320 and 1440 per 100,000 in developing nations of Africa and Southeast Asia, respectively (Kotloff et al., 2013; Majowicz et al., 2010; Petri et al., 2008). *Salmonella* enteritis is a major cause of malnutrition and growth retardation, two major complications of diarrhoeal disease (Petri et al., 2008). Protection against *Salmonella* infection involves coordination of innate and adaptive immune responses involving helper $CD4^+$ T cells, and to a lesser extent cytotoxic $CD8^+$ T cells and antibody production (Griffin & McSorley, 2011; Salerno-Goncalves, Fernandez-Vina, Lewinsohn, & Sztein, 2004). Antibiotic treatment of non-typhoidal *Salmonella* infection has a minimal impact on diarrhoea, and while new vaccines are improving protection, there remains a strong need for novel therapies (Anwar et al., 2014; Onwuezobe, Oshun, & Odigwe, 2012). Nutritional therapies that have shown promise for controlling salmonellosis include dietary fructooligosaccharides, short chain fatty acids (SCFA), barley, and rice hull extract in animal models (Bovee-Oudenhoven, ten Bruggencate, Lettink-Wissink, & van der Meer, 2003; Boyen et al., 2008; Kim, Kang, Park, Nam, & Friedman, 2012; Michiels et al., 2012; Pieper et al., 2012) as well as green banana and pectin, and dietary lactic acid fermented cereal gruel in humans (Kingamkono, Sjogren, & Svanberg, 1999; Rabbani et al., 2001). Though multiple interventions have been tested, little is known about immune responses following nutritional intervention. Interestingly, studies in weaning piglets demonstrated that protection following arginine supplementation resulted in reduced serum C reactive protein as well as (systemic or local) tissue expression of inflammatory biomarkers Myd88, TLR4, TLR5, NFκB and TNF-α following systemic *Salmonella* infection (Y. Chen et al., 2012). Recent studies from our laboratory have highlighted the potential of bran from the rice variety, "Neptune," to reduce *Salmonella* colonization using *in-vitro* and mouse models. In this case, protection is associated with increased native gut *Lactobacillus* numbers and reduced production of systemic proinflammatory cytokine production (Henderson, Kumar, Barnett, Dow, & Ryan, 2012; Kumar et al., 2012).

Rice is a global staple food crop and is thought to account for one fifth of the calories consumed worldwide (Sharif, Butt, Anjum, & Khan, 2014). Therefore, rice bran is presently available in most regions of the world, but is typically discarded as a by-product of rice polishing. Rice bran contains a number of bioactive components and has been shown to have a beneficial impact on numerous diseases including colon cancer, heart disease, and *Salmonella* infections (Forster et al., 2013; Henderson, Ollila et al., 2012; Jariwalla, 2001; Kim, Park, Lee, Nam, & Friedman, 2013, 2014; Kumar et al., 2012; Kuriyan, Gopinath, Vaz, & Kurpad, 2005). Additional studies have shown other benefits including antioxidant, antitumour, anti-atherosclerosis, diabetes control, and anti-allergy potential (Cheng et al., 2010; Forster et al., 2013; Goufo & Trindade, 2014; Henderson, Ollila et al., 2012; Nicolosi, Ausman, & Hegsted, 1991; Oka et al., 2010). A number of genetically and agronomically diverse rice varieties have been developed resulting in different grain sizes, aromas and nutritional qualities (Marr, Batten, & Blakeney, 1995; Rutger et al., 2004; Saenchai, Prom-u-thai, Jamjod, Dell, & Rerkasem, 2012; Schaeffer, Sharpe, & Dudley, 1994), and protection against plant diseases and pests (Fitzgerald, McCouch, & Hall, 2009; Huang et al., 2010; Khush, 1997; Ni, Colowit, & Mackill, 2002; Zhu et al., 2000). Varietal differences in bioactivity of rice bran have been observed in antitumour capacity (M. H. Chen, Choi, Kozukue, Kim, & Friedman, 2012; Forster et al., 2013). In the context of *Salmonella*, evidence supporting the importance of varietal differences has also been demonstrated. For instance, carbohydrate levels amongst barley varieties impacted *Salmonella* shedding from pigs, and rice bran extracts from Sung-Yod and Jasmine rice varieties had differential *in-vitro* antimicrobial capacity against *Salmonella* (Kondo, Teongtip, Srichana, & Itharat, 2011; Pieper et al., 2012).

The ability of rice bran to influence immunity has also been demonstrated. Feruloylated oligosaccharides from rice bran activated bone marrow derived dendritic cells by increasing CD40, CD86 and MHC-II expression in a TLR-4 and TLR-2 dependent fashion (Lin et al., 2014). In addition, rice bran oil consumption enhances systemic B cell proliferation and Th1 cytokines while suppressing Th2 cytokines and IgE (Sierra et al., 2005). Furthermore, our laboratory demonstrated that rice bran modulates intestinal immunity by increasing IgA production (Henderson, Kumar et al., 2012; Yang et al., 2014). In addition, rice varieties have been shown to possess disparate antioxidant potential, suggesting differential ability to neutralize reactive oxygen species generated during acute inflammation (M. H. Chen et al., 2012; Iqbal, Bhanger, & Anwar, 2005; Nam et al., 2006). The implications for differential effects of rice bran from diverse varieties on mucosal immunity and subsequent disease outcomes have not been previously investigated. In the current study we hypothesized that rice bran from diverse rice varieties would differentially modulate mucosal immune-mediated protection against *Salmonella* colonization. Dietary metabolites and intestinal immune responses were associated with protective efficacy against *Salmonella* infection and provide novel evidence for rice bran as a practical weapon against enteric disease.

2. Materials and methods

2.1. *Rice varieties, heat-stabilized rice bran and mouse diet preparation*

Six rice varieties were obtained from the USDA-ARS (Dale Bumpers National Rice Research Center, Stuttgart, AR, USA) as previously described (Forster et al., 2013). The varieties were grown under conventional production practices common to the southern USA rice-growing region. The varieties Red Wells (red bran), IL121-1-1 (red bran), and Jasmine 85 (brown bran) were produced in Beaumont, TX in 2009 while IAC600 (black/purple bran) was produced at the same location in 2007. The varieties Wells (brown bran) and Shufeng 121 (brown bran) were