Effects of dietary β-glucans supplementation on cytokine expression in porcine liver


College of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

ABSTRACT: As dietary supplementation with β-glucans can stimulate the innate immune response in the porcine gastrointestinal system (GIT), the aim of this study was to determine if the effects of β-glucan supplementation extend beyond the GIT to systemic levels. Hence, the effects of dietary supplementation of β-glucans derived from Laminara digitata, Laminara hyperborea, and Sacharomyces cerevisiae on cytokine expression in the porcine liver with or without ex vivo lipopolysaccharide (LPS) challenge were examined. No significant differences in gene expression were observed in the unchallenged liver tissue, but differences were observed in all supplementation groups in the LPS challenged tissue. Relative to the basal diet, IL-6 (P < 0.05) was less expressed in the S. cerevisiae supplementation group, IL-6 (P < 0.05) and TLR-4 (P < 0.05) were less expressed in the L. digitata supplementation group, and IL-10 (P = 0.06) and IL-1α (P = 0.02) were more expressed in the L. hyperborea supplementation group. There was a ~3-fold increase in both IL-10 and IL-1α in the liver samples of L. hyperborea relative to the L. digitata supplementation groups (P < 0.01). The results indicate that supplementation with β-glucans from both yeast and seaweed sources have systemic effects evidenced by changes in cytokine expression in the liver in response to LPS challenge; however, the cytokines affected varied according to the source of the β-glucan.

Key words: β-glucans, Laminara digitata, Laminara hyperborea, liver, Sacharomyces cerevisiae

INTRODUCTION

β-Glucans are abundant in the cellular walls of microorganisms such as yeast and also from plant sources such as mushrooms, seaweed, oat (Avena sativa), and barley (Hordeum vulgare). Variation in the distribution and length of the glucose side chains alters the biochemical and solubility properties of these complex polysaccharides in a manner largely dependent on their source. This variation in β-glucan structure is also reported to influence their immunomodulatory properties both in vitro and in vivo (Brown and Gordon, 2003). Seaweed-derived laminarins act as a source of dietary fiber and also have been reported to have antibacterial and anticytotoxic properties (Gupta and Abu-Ghannam, 2011). The β-glucans derived from L. digitata are water soluble and contain small numbers of β-(1-6)-linked side chains; in contrast, L. hyperborea-derived β-glucans are water insoluble and only contain linear β-(1-3)-linked residues (Read et al., 1996). β-Glucans derived from S. cerevisiae are composed of mainly branched β-(1-3)-glucan (85%) and contain 3% β-(1-6)-glucosidic interchain linkages (Manners et al., 1973).

Reports on the immunomodulatory effects of β-glucans are variable and difficult to consolidate. The main variables influencing outcome include (i) the source of the β-glucan extracts, (ii) whether administration was parenteral (intravenous or subcutaneous) or dietary, (iii) if the study was conducted in vitro or in vivo, and finally (iv) the tissue type being studied (Volman et al., 2008). Of most relevance to this study are the dietary supplementation studies. Oral administration of watersoluble β-glucans has been reported to translocate from the gastrointestinal system (GIT) in to the systemic system, generating modest increases in sera levels of IL-6 and IL-12 in rats (Rice et al., 2005). Increases in IL-6 and TNF-α in the blood of weaned pigs following lipopolysaccharide (LPS) challenge has also been reported following oral administration of S. cerevisiae (Li et al., 2006). We have previously demonstrated the immunomodulatory effects of β-glucans in the porcine colon (Sweeney et al., 2012). The objective of this study therefore was to compare the effects of dietary supplementation with β-glucans derived from...
L. digitata, L. hyperborea, and S. cerevisiae on cytokine gene expression in both ex vivo LPS challenged and non-LPS challenged liver.

MATERIALS AND METHODS

Experimental Groups

Forty-nine-day-old pigs with an initial body weight of 15.3 kg (SD = 1.32 kg) were allocated to one of the following 4 dietary groups (n = 8 per group): (i) basal diet (BD), (ii) BD + β-glucans from L. hyperborea, (iii) BD + β-glucans from L. digitata, and (iv) BD + β-glucans from S. cerevisiae. The basal diet was a wheat (Triticum aestivum)-based standard weaning pig formulation. The β-glucans were included at 250 mg/kg in the diets. All details of experimental design, diets, and animal management are outlined previously (Sweeney et al., 2012).

In vitro Immunological Treatment of Tissues

Tissue sections of liver (1 cm³) were placed in 1 mL Dulbecco’s modified Eagles medium (Gibco, In Vitrogen Corp., San Diego, CA) in the presence or absence of LPS 10 μg/mL (Escherichia coli serotype O111:B4; Sigma L5293, Sigma Chemical Inc., St. Louis, MO) and were incubated at 37°C for 90 min. Lipopolysaccharide stimulates an inflammatory response in tissues. The tissue was then removed and stabilized in RNAlater (Ambion Inc., Austin, TX) and stored at –70°C.

RNA Isolation, cDNA Synthesis, and Quantitative PCR

RNA isolation, cDNA synthesis, and quantitative PCR were carried out as previously described (Sweeney et al., 2012). The targets included tumor necrosis factor alpha (TNF-α), interleukins (IL-10, IL-8, IL-6, IL-4, IL-1α, and IL-17α), interferon gamma (INFγ), and Toll-like receptor 4 (TLR-4). The expression of porcine β-glucan receptor (CLEC-7) was also measured using the following unpublished primer set: Forward: 5′-GCACATCATTAGATCTTCGGA-3′ and reverse: 5′-GGAGGCTGTCATCTCTCCAGGAGA-3′. The geometric mean of two previously published endogenous controls glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and beta-2 microglobulin (B2M) were used to normalize the target genes according to the method previously described (Ryan et al., 2010). Normalized relative quantities were analyzed using the GLM procedure in SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Results are presented on Table 1. There were no differences in the expression of any of the genes examined between the basal diet and the β-glucan supplemented diets in the absence of an LPS challenge (data not shown). However, a number of differences were observed following the LPS challenge: IL-6 was less expressed in the liver tissue of the S. cerevisiae supplementation group when compared to the basal diet (P = 0.05), IL-6 and TLR-4 were less expressed in the liver tissue of the L. digitata supplementation group relative to the basal diet (P = 0.04 and 0.017, respectively), and IL-10 and IL-1α were more expressed in the liver samples of the L. hyperborea supplementation group relative to the basal diet (P = 0.06 and 0.02, respectively). A ~3-fold increase in both IL-10 and IL-1α was observed in the liver samples of L. hyperborea relative to the L. digitata groups (P = 0.0008 and 0.0025, respectively).

DISCUSSION

No differences were observed in cytokine gene expression in the unchallenged liver of animals on any of the β-glucan supplemented diets. However, there were

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>L. digitata</th>
<th>L. hyperborea</th>
<th>S. cerevisiae</th>
<th>SE</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>1</td>
<td>1.09</td>
<td>1.33</td>
<td>1.59</td>
<td>0.02</td>
<td>ns</td>
</tr>
<tr>
<td>IL-10</td>
<td>1</td>
<td>0.48a</td>
<td>1.74a</td>
<td>1.05ab</td>
<td>0.01</td>
<td>**</td>
</tr>
<tr>
<td>IL-8</td>
<td>1</td>
<td>0.51</td>
<td>1.43</td>
<td>1.15</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>1a</td>
<td>0.37b</td>
<td>0.57ab</td>
<td>0.35b</td>
<td>0.01</td>
<td>*</td>
</tr>
<tr>
<td>IL-4</td>
<td>1</td>
<td>1.63</td>
<td>0.70</td>
<td>0.25</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td>IL-1α</td>
<td>1</td>
<td>0.77a</td>
<td>2.45a</td>
<td>1.28ab</td>
<td>0.01</td>
<td>*</td>
</tr>
<tr>
<td>IL-17α</td>
<td>1</td>
<td>2.79</td>
<td>1.59</td>
<td>1.41</td>
<td>0.002</td>
<td>ns</td>
</tr>
<tr>
<td>INFγ</td>
<td>1</td>
<td>2.30</td>
<td>1.41</td>
<td>4.76</td>
<td>0.02</td>
<td>ns</td>
</tr>
<tr>
<td>CLEC-7</td>
<td>1</td>
<td>0.75</td>
<td>0.80</td>
<td>1.05</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td>TLR-4</td>
<td>1</td>
<td>2.14b</td>
<td>0.85a</td>
<td>0.67b</td>
<td>0.01</td>
<td>*</td>
</tr>
</tbody>
</table>

a,bValues with different superscript within a row are significantly different (P < 0.05).

1ns = nonsignificant.

*P < 0.05; **P < 0.01.
significant cytokine responses in the liver following an LPS challenge, with the cytokine response varying with the source of β-glucan. IL-6 was significantly reduced in the liver in both the L. digitata and S. cerevisiae supplementation groups relative to the basal diet following LPS challenge. This is consistent with the view that β-glucans prime the immune system thereby altering subsequent responses to infection (Volman et al., 2008). It has been reported that stimulation of TLR-4 by LPS in the liver results in nuclear factor κB-mediated production of proinflammatory cytokines such as IL-6 by the Kupffer cells (Seki et al., 2002). A mouse (Mus musculus) model of sepsis has also demonstrated that soluble poly-[1-6]→D-glucopyranosyl-[1-3]→D-glucopyranose glucan induced significant decreases in IL-6 and IL-10 plasma levels and bacterial colonization of the liver (Newsome et al., 2011). From a therapeutic perspective it is desirable to have the ability to prime host defenses without contributing to a systemic inflammatory response. In this study it is interesting that both TLR-4 and IL-6 are less expressed in the LPS challenged liver in the L. digitata supplementation group, suggesting an attenuated response to LPS challenge.

Differences are evident between the L. digitata and L. hyperborea supplementation groups with respect to IL-10 and IL-1α expression in the liver, which is consistent with the view that there are differences in how these soluble and insoluble β-glucans are absorbed (Sweeney et al., 2012). In this study, differences were observed in L. digitata in terms of proinflammatory response to LPS challenge and fermentation in the colon relative to L. hyperborea and S. cerevisiae. The findings of this study indicate that supplementation with β-glucans is having systemic albeit differential effects. It is currently unclear if these effects are attributable directly to the β-glucans following their absorption and uptake within the liver or indirectly due to alterations in the gut microbiota brought about by the β-glucans.

**LITERATURE CITED**


