Dietary supplementation with mannanoligosaccharides and β-glucans in growing rabbits. 2 Gut barrier and intestinal microbiota after weaning


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ABSTRACT

The aim of this work was to determine the effect of yeast mannanoligosaccharides (MOS; Actigen, Alltech) and yeast β-glucans on gut barrier and intestinal microbiota in rabbits. A control diet (C) was formulated to meet rabbit growth requirements. G1 and G2 diets were obtained by supplementing C diet with β-glucans from Saccharomyces cerevisiae (100 and 200 mg/kg respectively), M1 and M2 diets were supplemented with mannanoligosaccharides (Actigen, Alltech) (400 and 800 mg/kg respectively), and GM diet was supplemented with 100 mg/kg of β-glucans + 400 mg/kg of MOS. Ninety six rabbits weaned at 32 days of age were randomly assigned to the six experimental diets and slaughtered 15 days after weaning. The type of diet did not affect villus length and goblet cell counts, but crypt depth was reduced at the highest levels of MOS and β-glucans supplementation. Both MOS and β-glucans increased specific sucrose activity (P = 0.001) and soluble protein concentration (P < 0.05) in jejunal mucosa; they also modified ileal and caecal microbiota and modulated the immune response. Compared with the control diet, the supplementation with 200 mg/kg of β-glucans (G2) and 800 mg/kg of MOS (M2) reduced the expression of iNOS in ileum. Rabbits fed the M2 diet showed also the lowest mRNA abundance of IL6. In appendix, rabbits fed G2 showed increased IL6 expression and those fed the M2 reduced the expression of INFγ. In conclusion, MOS and β-glucans changed intestinal microbiota and modulated the immune response by reducing the expression of some pro-inflammatory cytokines in ileum and caecum of weaned rabbits.

Key words: Yeast mannanoligosaccharides, yeast β-glucans, weaning rabbit, immunity, microbiota, jejunum morphology

INTRODUCTION

Mannanoligosaccharides (MOS) and β-glucans extracted from Saccharomyces cerevisiae are promising alternatives to antibiotic growth promoters (Yang et al., 2008; Bovera et al., 2012). They have shown beneficial effects on mucosal integrity and are capable of modulating the immune response and intestinal microbiota in chicken and pigs (Castillo et al., 2007; Yang et al., 2008). In rabbits, recent publications suggest that these prebiotics increase villus length (Mourao et al., 2006) and reduce coliforms count in the caecum (Bovera et al., 2012). Wu et al. (2011) observed that β-glucans improved maternal humoral immunity at late lactation by elevating serum Ig concentrations in does. However, information about the effect of MOS and β-glucans on gut barrier and intestinal microbiota in weaned rabbits is scarce. The aim of this work was to determine the effect of yeast MOS and β-glucans on gut barrier, intestinal microorganism and immune response in rabbits around weaning.
MATERIALS AND METHODS

Animals and experimental diets

Ninety six Hyplus rabbits (Grimald Frères, France) were weaned and randomly assigned to 6 experimental diets (16 animals per treatment). From weaning (32 days of age) to 46 days old (1634 ± 137 g LW), rabbits were fed ad libitum as follows: C (control diet without additives), G1 and G2 (control diet supplemented with 100 mg/kg or 200 mg/kg of β-glucans, respectively, extracted from Saccharomyces cerevisiae by the Instituto de Estudios Biofuncionales, Madrid), M1 and M2 (control diet supplemented with 400 mg/kg or 800 mg/kg of MOS, respectively, Antigen, Alltech, Spain) and GM (control diet supplemented with 100 mg/kg of β-glucans + 400 mg/kg of MOS). Ingredients and chemical composition of diets have been described by Tazzoli et al. (2012). All rabbits were housed individually and kept under controlled environmental condition.

Slaughter and samples collection

During two consecutive days (at 45 and 46 days of age), 96 rabbits (16/diet) were slaughtered by cervical dislocation. Immediately, 10 cm from middle jejunum were collected. The first 5 cm were washed with saline solution (0.9 % NaCl), frozen in dry ice and stored at -80°C until determination of sucrase activity. The other 5 cm were stored in 10% buffered formalin solution (pH: 7.2-7.4) for analysis of mucosa morphology and goblet cell count. One gram of caecal and ileal content was collected and placed in sterile tubes containing 3 ml of ethanol solution (98%) and stored at 4°C for microbial analysis. For cytokine analysis, only rabbits from diets C, G2 and M2 were used (6 rabbits/diet). In these animals, 3 cm of the ileum (between ileo-caecal junction and Payer patch) and the vermiciform appendix were taken. All samples were washed with saline solution (0.9 % NaCl), longitudinally opened and mucosal scrapings placed in vials containing 2 ml of RNA-later (Ambion) and stored at -80°C until immunological analyses.

Analytical methods

Sucrase activity (EC 3.2 1.48) was determined using sucrose as substrate and following the procedure described by Gómez-Conde et al. (2007). Glucose liberated was measured by using a commercial kit (Randox, Gluc-PAP). The soluble protein concentration was determined on jejunum homogenates using a commercial kit (Thermo scientific, Pierce® BCA Protein Assay Kit). To evaluate the jejunal mucosa morphology, goblet cell count, and gut microbiota samples were processed according to Gómez-Conde et al. (2007). To study the immune response, the expression of selected cytokines was assessed by real time RT-PCR with the ABI Prism 7300 Sequence Detector using the SYBR green dye (Applied Biosystems). Total RNA was isolated from mucosal scrapings using Trizol and the GeneElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich,) with an additional on-column DNase I digestion step. To synthesize cDNA, the High-Capacity cDNA Archive Kit was used (Applied Biosystems) according to manufacturer’s specifications. The relative abundance of INOS, INFγ, TNFα, IL-2, IL-6, IL-8 and IL-10 mRNA for appendix and of iNOS, IL-6 and IL-10 for ileum was studied. The specific primers for rabbit housekeeping (GADPH), IFN-γ, IL-10 and TNF-α were selected according to Menoyo et al. (2010).

Statistics analysis

Data of mucosa morphology and enzymatic activity was analyzed as a completely randomized block design with a diet as the main source of variation by using a Mixed procedure of SAS (1999). Contrasts were used to compare means. For each contrast, the changes in gene expression resulted from the comparison of the first treatment group relative to the second, following the model described by Steibel et al. (2009) using the SAS Mixed procedure. For genes displaying efficiencies different from 2 (E≠2), Ct values were adjusted according to the model described by Steibel et al. (2009).
RESULTS AND DISCUSSION

The type of diet did not affect villus length and the number of goblet cells per villus (Table 1), showing normal values for 45-d-old rabbits. However, both additives included at the highest levels reduced the crypt depth (P < 0.05). These results agree with those reported in pigs and broiler (Castillo et al., 2007, Yang et al., 2008), but contrast with those of Mourao et al. (2006) in rabbits. These latter reported that MOS supplementation increased the length of villus in ileum compared to a no supplemented diet. This difference between studies might be due to differences of health status (3.5 vs. 8.5% mortality during fattening period). The good health of the animals in the present study is compatible with a good gut morphology and lower crypt cell turnover. The β-glucans and MOS supplementation increased the specific sucrase activity and soluble protein concentration compared to the control diet (P < 0.05), which suggests an improvement of enterocyte maturity and/or functionality as in other species. Chicken fed diet supplemented with MOS showed higher specific activity of maltase and alkaline phosphate, which is used as indicator of intestinal maturity (Iji et al., 2001; Yang et al., 2008). This effect can also be explained by changes of intestinal microbiota, as suggested by Yang et al. (2008) who reported an increase of Lactobacillus in broilers fed MOS. This increase seems to improve the intestine digestive enzyme activity (Sissons, 1989). Dietary treatments did not affect the gene expression of cytokines in appendix, except for IL6, which increased (P < 0.05) in animals fed G2, and INFγ, which displayed a lower expression pattern (P < 0.05) in rabbits fed M2 (Figure 1). Compared to the control, rabbits fed G2 and M2 diets reduced the expression of INOS in ileum and M2 also reduced the expression of IL6. Since rabbits affected by ERE showed a higher mRNA abundance of IL6 and INOS in ileum relative to healthy animals (Menoyo et al., 2010), these additives could be useful to reduce the pro-inflammatory response of infected animals. However, this point needs to be deeply studied. In this trial we did not characterize or count the intestinal microbiota but the supplementation with MOS and β-glucans led to a modification of both ileal and caecal microbiota (Figure 2). In conclusion, MOS and β-glucans changed intestinal microbiota and modulated the immune response by reducing the expression of some pro-inflammatory cytokines in ileum and caecum of rabbits around weaning.

Table 1: Effect of diet on morphology, goblet cell counts and sucrase activity of jejunal mucosa in 45 days old rabbits.

<table>
<thead>
<tr>
<th>Diet</th>
<th>C</th>
<th>G1</th>
<th>G2</th>
<th>M1</th>
<th>M2</th>
<th>GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C vs. Others</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>G1 vs. G2</td>
<td>65.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>M1 vs. M2</td>
<td>8.71</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>G1+G2 vs. M1+M2</td>
<td>0.62</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.09</td>
</tr>
<tr>
<td>G1+M1</td>
<td>1066</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>G1 vs. M2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C vs. GM</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.038</td>
<td>NS</td>
</tr>
<tr>
<td>Soluble protein (μg/100mg tissue)</td>
<td>8.30</td>
<td>8.75</td>
<td>8.53</td>
<td>8.54</td>
<td>8.72</td>
<td>8.39</td>
</tr>
</tbody>
</table>

1 C control diet without additive. G1: diet C with 100 mg of β-glucans/kg. G2: diet C with 200 mg of β-glucans/kg. M1: diet C with 400 mg of MOS/kg. M2: diet C with 800 mg of MOS/kg. MG: diet C with 100 mg of β-glucans+ 400 mg of MOS/kg. 2 NS: P > 0.10. 3 measured at 37°C during 30 min, μmol of glucose/g of protein.
Figure 1: Relative gene expression of selected cytokines in animals fed G2 (200 mg of β-glucans/kg) and M2 (800 mg of MOS/kg) diets compared with rabbits fed C diet

Figure 2: Effect of diet on similarity rate of PCR-RFLP banding pattern of the ileal (A) and caecal (B) microbiota (SR, expressed in percentage) in 45-d-old rabbits. (B: control diet, G2: diet C with 200 mg of β-glucans/kg; M2: diet C with 800 mg of MOS/kg; MG: diet C with 100 mg of β-glucans + 400 mg of MOS/kg). The number of each animal is its identification.
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REFERENCES


