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NATIVE AND SATURATED BOVINE LACTOFERRIN MODULATE THE EXPRESSION OF TOLL-LIKE RECEPTORS (TLRS) IN MICE TREATED WITH ANTIBIOTICS

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Introduction: The gut microbiota plays a fundamental role in protection against pathogen infections but also in the regulation of the immune system. The antibiotic administration can result in gut microbiota alterations and changes in the effectiveness of innate immune responses. As microbiota composition changes, the altered community will present different microbial-associated molecular patterns to Toll-like receptors (TLRs), which can cascade down through various immune responses, including changes in the expression of TLR receptors [1].

In this context, finding ingredients that can modulate the immune system is becoming a major focus of interest to produce functional foods. Bovine lactoferrin is a milk protein with many biological properties, including antibacterial and immunomodulatory activity [2].

Aims & Methods: The objective was to determine the effects of native (nLf) and saturated (sLf) bovine lactoferrin on TLR receptor expression of colon from mice treated or untreated with the antibiotic clindamycin, to evaluate its potential to be added in functional foods.

Male C57BL/6 mice of 8 weeks old were randomly divided into six groups (n=5 per group): vehicle, clindamycin (Clin), native bovine lactoferrin (nLf), nLf + clindamycin (nLf_Clin), iron-saturated bovine lactoferrin (sLf) and sLf + clindamycin (sLf_Clin). Vehicle received saline orally for 10 days. Clin was gavaged for 10 days with saline and on day 4 received a single IP injection of 200 µg of clindamycin. nLf and sLf were gavaged for 10 days with 35 mg of nLf or sLf. The groups nLf_Clin and sLf_Clin were gavaged with nLf or sLf and on day 4 received an injection of clindamycin.

The gene expression (mRNA) of TLR1-9 receptors was determined in the colon from mice by RT-PCR and relative expression levels of genes were calculated using the $2^{-\Delta\Delta CT}$ method.

Results: The expression of TLR2, TLR4, TLR5 and TLR6 was not modified in any of the treated groups. The expression of TLR1 was increased in sLf, nLf_Clin and sLf_Clin treated groups, indicating that the effect of sLf on TLR1 expression was maintained despite the treatment with Clin. The expression of TLR8 and TLR9 decreased in Clin, nLf and nLf_Clin groups. However, the levels of these receptors in sLf_Clin were similar to Vehicle, demonstrating the immunomodulatory capacity of sLf to restore these receptor levels, in a situation of intestinal dysbiosis.

Finally, respect to TLR3 and TLR7, only the group of mice treated with sLf_Clin showed an increase in the expression of this receptor.

Conclusion: Saturated bovine lactoferrin can restore the expression levels of TLR8 and TLR9 in conditions of intestinal dysbiosis induced by antibiotics.

These results confirm the immunomodulatory properties of lactoferrin, which have great interest for the design of functional foods.

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Disclosure: Nothing to disclose.

P0204

ASSESSMENT OF GUT MICROBIOTA IN IBS PATIENTS AFTER A 4-WEEK STARCH-AND SUCROSE-REDUCED DIET SHOWS INCREASED BETA DIVERSITY, UNALTERED ALPHA DIVERSITY AND SPECIFIC CHANGES IN GENERA

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Introduction: We have previously reported that IBS patients exhibit improved gastrointestinal (GI) symptoms following a starch- and sucrose-reduced diet (SSRD)[1]. We aimed to examine the effect of the diet on gut microbiota.

Aims & Methods: IBS patients were randomized to a 4-week SSRD intervention (n=80) or control group (n=25); habitual diet). At baseline and 4 weeks, fecal samples and 4 day-dietary records were collected, and participants filled out GI symptom questionnaires, i.e., the VAS-IBS, IBS-SSS and Rome IV questionnaires. DNA was extracted from the fecal samples and analyzed through 16S rRNA gene amplicon sequencing, from the variable regions v1-v2.

Results: Seven different phyla were identified. The most dominant phyla in the intervention and control group both pre- and post-intervention were Bacteroidetes (relative abundance 55-60%) and Firmicutes (28-32%). The most notable change in phyla distribution during the intervention was an increase in the relative abundance of Proteobacteria in the intervention group, from 6.8% to 11.0%. There were no significant changes in alpha diversity in either group following the SSRD intervention. Beta diversity increased significantly in the intervention group (Permanova, $p<0.001$), but not in controls (data not shown). Specific genera, including the *Eubacterium eligens* group, *Lachnospiraceae UCG-001*, *Lachnospira* and *Enterobacter* increased significantly in the intervention group (log2 fold change: *Eubacterium eligens* group: 3.1; *Lachnospiraceae UCG-001*: 2.2, *Lachnospira*: 1.6 and *Enterobacter*: 1.4; p<0.001 for all). Weak correlations were identified between decreases in starch, carbohydrate and disaccharide intakes and increased beta diversity in the intervention group (table 1). No correlations could be identified between changes in beta diversity and total IBS-SSS or individual GI symptom scores.

Nutrient	R^2	*p*
Protein (E%)	0.0660	0.009
Carbohydrates (g)	0.0491	0.028
Starch (g)	0.0681	0.013
Starch (E%)	0.0451	0.025
Disaccharides (g)	0.0577	0.014

E%= energy percentage. g=gram. Permanova. R^2 = correlation coefficient. $p < 0.05$ was considered significant.

Table 1. Correlations between change in beta diversity and changes in nutrient intakes in the intervention group