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### ► To cite this version:

Gaëlle Boudry, Agnès Jamin, Livie Chatelais, Catherine Michel, Isabelle Luron Le Huërou-Luron. Is the colon submitted to nutritional programming? Delayed effect of a high protein formula on colonic physiology in low birth weight piglets. 43. Annual Meeting of the European Society for Paediatric Gastroenterology, hepatology and Nutrition, Jun 2010, Istanbul, Turkey. hal-02754557

**HAL Id: hal-02754557**

**<https://hal.inrae.fr/hal-02754557>**

Submitted on 3 Jun 2020

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## Is the colon submitted to nutritional programming? Delayed effect of a high protein formula on colonic physiology in low birth weight piglets.

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### Background:

Numerous neonatal practices are likely to affect intestinal microbiota implantation. However, the intense period of interplay between colonic microbiota, epithelial and immune cells during the neonatal period is believed to shape their feature for the entire life. By increasing the colonic supply of non digested proteins and peptides, protein-enriched formulae could affect the intestinal microbiota, particularly in neonates suffering from intrauterine growth restriction who exhibit lower digestive capabilities. Using low birth weight piglets, we have therefore investigated whether a high protein formula would modify both the colonic microbiota implantation and the development of the immune and non-immune colonic barrier.

**Methods:** Low birth weight piglets were fed from day 2 to 28 either a normoproteic (NP) or a high protein (HP, +40% protein) formula and then the same diet for both groups until day 160. Immediate (d28) and delayed (d160) consequences on colonic microbiota (Q-PCR), *ex vivo*-permeability (Ussing chambers), cytokines profiles (RT-PCR) and immune cell density (IHC), and response to oxidative (0.1mM NH<sub>2</sub>Cl in Ussing chambers) and inflammatory stresses (explants culture with 0 to 200 µg/mL of LPS) were evaluated.

**Results:** During the neonatal period (d28), the HP formula modified the colonic microbiota by increasing the percentages of *Bacteroides* (17±3 vs 10±3%, P<0.05) and of bacteria from the *Clostridium leptum* cluster (1.8±0.5 vs 0.9±0.3%, P<0.05) compared to NP piglets. Permeability to macromolecules tended to be higher in HP compared to NP piglets (104±46 vs 17±7 ng/cm<sup>2</sup>/h, P<0.06). Moreover IL-1β and TNFα mRNA levels were reduced (0.5±0.2 vs 1.2±0.1 and 2.3±0.3 vs 3.8±0.8, respectively, P<0.05) while T cells and myeloid cells densities were increased (X 1.5 and 1.9, P<0.05) in HP piglets.

Later in life (d160), no impact was noticed on colonic physiology in males. However, HP females had lower percentages of *Faecalibacterium prausnitzii* compared to NP ones (0.9±0.4 vs 2.2±0.4%, P<0.05). Their colons also displayed higher permeability following oxidative stress (X 6.5, P<0.05) and higher secretion of IL8 and IL-1β in response to LPS (X 2.4 with 200µg/mL LPS, P<0.05).

**Conclusion:** The protein enrichment modified the post-natal development of colonic microbiota and immune and non-immune barrier. Later in life, in females, it was associated with some features which have been related to Crohn's disease (reduced percentage of *F. prausnitzii*, higher sensitivity to

oxidative and inflammatory stresses). This study clearly demonstrates for the first time the presence of long-term effect of neonatal nutrition on colonic physiology.