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## **Supplementation of the maternal diet with alpha-linolenic acid (ALA) modifies the development of the enteric nervous system with functional consequences on intestinal permeability in newborn piglets**

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The enteric nervous system (ENS) participates in the regulation of various mucosal functions such as intestinal motility and secretion but also permeability. The ENS shares embryologic similarities with the CNS whose perinatal development is known to be modulated by n-3 polyunsaturated fatty acids (PUFA). We hypothesized that n-3PUFA in the maternal diet modify the development of the ENS, by analogy with the CNS, and have consequences on the regulation of jejunal permeability of newborn piglets.

The intestinal permeability was measured on 28 day-old piglets nursed by sows who had been fed either a lard-based diet (LAR, ALA = 3.5% of total fatty acid) or a linseed oil based-diet (LSO, ALA= 28.3%) during gestation and lactation. Basal jejunal permeability to FITC-dextran 4000 (FD4) was evaluated in Ussing chambers. Different antagonists of the ENS were used to determine the nervous regulation of intestinal permeability. Additional intestinal tissue were collected to evaluate cholinergic neurons percentages in the ENS by immunohistochemistry and to analyze the expression of tight junction protein and choline acetyl transferase (ChAT) by Western blot and RT-PCR. Inflammatory consequences were also evaluated by analyse of cytokines mRNA in the tissue.

Basal jejunal permeability to FD4 was higher in LSO compared to LAR piglets ( $621 \pm 55$  vs  $341 \pm 45$  ng/cm<sup>2</sup>/h,  $P < 0.01$ ). The protein or mRNA expressions of tight junction did not differ between the two groups of piglets. In the LAR group, TTX and VIPra increased jejunal permeability (+ 25%,  $P < 0.05$  and +76%,  $P < 0.01$ , respectively), suggesting a VIPergic tonus. On the contrary, in the LSO group, VIPra did not modify basal permeability but TTX and atropine/hexamethonium decreased FD4 flux (-20%,  $P = 0.07$  and -30%,  $P < 0.05$ , respectively), suggesting that the high permeability was due to a cholinergic tonus. Moreover, the proportion of cholinergic neurons in the ENS of LSO piglets was greater than in LAR piglets ( $22.9 \pm 1.2\%$  for LAR vs  $26.6 \pm 0.9\%$  for LSO,  $P < 0.05$ ) and the mRNA relative expression of ChAT was greater in the LSO group compared with the LAR group ( $1.9 \pm 0.6$  vs  $1.1 \pm 0.6$ ,  $P < 0.05$ ). Despite the increase in permeability the cytokine profiles (IL-1 $\beta$ , IL10, TGF- $\beta$ ) did not differ between the two groups.

In conclusion, supplementation of the maternal diet with ALA modified the development of the ENS and the nervous regulation of jejunal permeability, with the induction of a cholinergic tonus. The consequences of such differences on the maturation of the gut immune system of newborn warrant further investigations.