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# Herbivore nutrition supporting sustainable intensification and agro-ecological approaches

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### Milk restricted lambs have greater colonic butyrate and a transitory modified anti-oxidant status

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Take home message Milk restriction of suckling lambs promotes an anti-inflammatory response in the colon and a transitory modified anti-oxidant status.

**Introduction** Nutritional interventions during early life may affect the development of the gastrointestinal tract and the maturation of the immune system, compromising metabolism and health status in the later stages of life (Bach, 2012). Thus, the aim of this study was to investigate the impact of moderate milk restriction during the suckling period of Assaf lambs on colonic parameters and long-term effects on biochemical parameters related to inflammatory response and oxidative stress.

Material & methods Forty Assaf lambs (average BW 4.73 kg) were penned individually, twenty of them were fed milk replacer (200 g dry matter/L) ad libitum (ADL; 192 mL/kg LBW) whereas the other group (restricted, RES) only received 120 mL/kg LBW. Eight lambs from each group were slaughtered at 35-days-old and colonic samples were collected for volatile fatty-acid analysis, analysis of microbial composition (qPCR), study of gene expression of toll-like receptors and cytokines (qRT-PCR), quantification of secretory IgA (ELISA), morphometric analysis (thickness of colonic layers) and immunohistochemistry examination (e.g., lymphocytes infiltrated in the lamina propria). The rest of the lambs (12 ADL vs. 12 RES) were weaned progressively, offered alfalfa hay and a starter concentrate ad libitum during 6 weeks and then a total mixed ration (TMR) ad libitum until the end of the experiment. Animals were blood sampled at the end of the feed restriction period (1st month) and once when the ewe lambs were being fed exclusively the TMR (6th and 8th months). Colonic parameters were analysed using GLM procedure of SAS, with the milk replacer intake level as the only source of variation. Biochemical parameters were analysed as a repeated measures design using the MIXED procedure of SAS.

Results & discussion During the suckling period, a greater proportion of butyrate (4.47 % vs. 7.07 %, P=0.014) and a trend towards an increased expression of the anti-inflammatory cytokines TGF- $\beta$  (P=0.055) and IL-4 (P=0.081) were found in the colon of RES lambs. These results could be associated with an increase of *Butyrivibrio spp.* and the lower relative abundance of *Prevotella spp.* observed in the colonic mucosa of restricted lambs. The rest of colonic parameters and two positive acute-phase proteins (*e.g.*, haptoglobin and ceruloplasmin) related to inflammatory response were not affected by milk replacer restriction, but levels of albumin, paraoxanase and total antioxidant activity (FRAP) were significantly reduced in RES lambs at the end of the 1<sup>st</sup> month of life, probably due to a decreased nutrient intake (Table 1).

**Table 1** Biochemical parameters of lambs fed *ad libitum* (n=12, ADL) or restricted (n=12, RES) during the suckling period.

	1st month		6 <sup>th</sup> month		8th month		P-value				
	ADL	RES	ADL	RES	ADL	RES	$SED_1 \\$	$SED_2$	T	D	T*D
Haptoglobin, g/L	0.51	0.56	0.27	0.40	0.17	0.39	0.170	0.177	0.450	0.341	0.893
Ceruloplasmin, µmol/L	3.86	4.24	2.70	2.97	2.53	2.76	0.445	0.308	0.514	< 0.001	0.967
Paraoxanase, U/L	173 <sup>b</sup>	134ª	159 <sup>b</sup>	149 <sup>ab</sup>	166 <sup>b</sup>	152 <sup>b</sup>	12.5	4.86	0.106	0.455	0.012
ROM, mg/100 mL	20.5	20.8	15.3	16.3	14.3	15.3	1.74	1.24	0.672	< 0.001	0.951
FRAP, μmol/L	155 <sup>b</sup>	125ª	134 <sup>a</sup>	133ª	134ª	131a	5.77	5.32	0.065	0.289	0.018
SOD, U/L	1.03	0.962	1.25	1.22	1.34	1.46	0.125	0.086	0.952	< 0.001	0.500
Retinol, ug/100 mL	22.0	22.5	53.3	45.2	58.0	49.1	3.92	3.21	0.174	< 0.001	0.286
Tocopherol, ug/mL	1.16	1.42	0.782	0.700	0.681	0.620	0.115	0.071	0.732	< 0.001	0.036
Albumin, g/L	$33.6^{b}$	30.4 <sup>a</sup>	37.1 <sup>d</sup>	35.8°	$36.7^{\text{cd}}$	36.0°	0.39	0.36	< 0.001	< 0.001	0.005

SED<sub>1</sub>, standard error of the difference to compare dietary treatments; SED<sub>2</sub>, standard error of the difference to compare days; T, dietary treatment; D, day; T\*D, interaction between treatment and day.

**Conclusion** Moderated milk replacer restriction of artificially reared suckling lambs promoted both, an anti-inflammatory response of the colon and a transitory modified antioxidant status that was corrected after weaning.

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