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Abstract. Bacteria firmly attached to the gastrointestinal epithelium during early life show a significant impact on nutrient processing, immune-stimulation, health and feed efficiency traits during the entire life of lambs. Thus, the aim of the present study was to describe the changes in the colonic epimural bacterial community of fattening lambs promoted by different levels of nutrition during the suckling phase trying to shed some light on the underlying mechanisms behind different feed efficiency traits. Twenty four merino lambs (average LBW 4.81 ± 0.256 kg) were used, twelve of them (*ad libitum*, ADL) being kept permanently with the dams whereas the other group (restricted, RES) was separated periodically from the dams and milk restricted. After weaning all the animals were penned individually, offered the same complete pelleted diet at a restricted level (35 g/kg LBW to ensure no differences of dry matter intake) and slaughtered with a target body weight of 27 kg. During the fattening period, lower gain:feed ratio (0.320 vs. 0.261, P < 0.001) was observed for the RES group. Additionally, an increment of *Prevotella sp.* was detected in the colonic epimural bacterial community of RES, whereas *Proteobacteria* was decreased. However, the colonic gene expression of toll-like receptors and cytokines (ΔCq), immunohistochemistry parameters (counts of lymphocytes T, B) and IgA levels (pg IgA/µg total protein) were not modified. In conclusion, the level of nutrition during the suckling phase promoted changes in feed efficiency traits and colonic epimural bacterial community that were not related to the immune response at this level.

Keywords. Feed efficiency – Lambs – Colon – Microbiota – Immunity.

Le niveau de nutrition des agneaux d'allaitement modifie la communauté bactérienne epimurale du colon et les traits d'efficacité alimentaire lors de l'engraissement

Résumé. *Les bactéries fermement attachées à l'épithélium gastro-intestinal au début de la vie montrent un impact significatif sur l´utilisation des nutriments, de stimulation immunitaire, de santé et d'efficacité alimentaire pendant toute la vie des agneaux. L'objectif de la présente étude était de décrire les changements dans la communauté bactérienne epimurale du colon d'agneaux d'engraissement promu par différents niveaux de nutrition pendant la phase d'allaitement en essayant de clarifier les mécanismes sous-jacents derrière différents traits d'efficacité alimentaire. Vingt-quatre agneaux mérinos (moyenne LBW 4,81 ± 0,256 kg) ont été utilisés, douze d'entre eux (ad libitum, ADL) étant maintenus en permanence avec les mères tandis que l'autre groupe (restreint, RES) a été séparé périodiquement des mères. Après le sevrage, tous les animaux ont été hébergés individuellement, ont reçu la même ration complète à un niveau restreint (35 g/kg LBW pour éviter toute différence de matière sèche ingérée) et abattu avec un poids corporel moyen de 27 kg. Les agneaux du groupe RES ont montré un taux de conversion inférieur à ceux du groupe ADL (0,32 contre 0,261, P<0,001). En plus, on a détecté une augmentation de* Prevotella sp. *dans la communauté bactérienne epimurale du colon des agneaux du groupe RES, alors que les protéobactéries ont diminué. Cependant, l'expression génique des récepteurs Toll-like et des cytokines (ΔCq) du colon, les paramètres d'immunohistochimie (comptage de lymphocytes T, B) et IgA (pg IgA / µg de protéines totales) n'ont pas été modifiés. En conclusion, le niveau de nutrition pendant la phase d'allaitement a favorisé les changements dans les traits d'efficacité d´alimentation et la communauté bactérienne épimurale du colon qui n'étaient pas liés à la réponse immunitaire à ce niveau.*

Mots-clés. *Efficacité d´alimentation – Agneaux – Colon – Microbiota – Immunité.*

I – Introduction

The Animal Task Force White Paper (2013) has identified improving animal feed efficiency and understanding of the interactions between nutrition, microbiome and immunity in the gut as priorities of research under the Horizon2020 strategy.

The epithelium of the gastrointestinal tract (GIT) is involved in a major part of the immune system and it is well-known that colonization of gut mucosal surfaces can be modulated by nutritional interventions during early life (Taschuk and Griebel, 2012). Moreover, once established, microbiota firmly attached to the GIT mucosa (epimural) seems to be more stable than that associated with GIT contents (Petri *et al*., 2013). Thus, manipulating epimural bacterial community in the first stages of life by nutritional management could promote long-term effects on immune response and/or the efficiency of utilization of nutrients along the whole life of the animals.

The aim of the present study was to assess whether bacterial colonization of colon mucosa is modified by the level of nutrition during the suckling period of merino lambs, hence promoting long-term effects on feed efficiency traits and colonic immune parameters during the fattening period.

II – Material and methods

Twenty four male merino lambs were used in the experiment. The lambs were stratified on the basis of live body weight at birth (average LBW 4.81 ± 0.256 kg), and then assigned randomly to one of two experimental treatments (n=12 per dietary treatment) during the suckling phase. The first group (*ad libitum*, ADL) was kept permanently with the dams, whereas the other (restricted, RES) was separated from the sheep from 9 to 18 h (dams were milked at 17 h and injected with oxytocin to remove alveolar milk). When lambs reached 13.5 kg of LBW they were weaned progressively until they weighed 15 kg. Then, all the animals were penned individually and offered the same complete pelleted diet (CPD) at a restricted level (35 g/kg LBW each day) to avoid differences in dry matter intake (DMI). After a fattening period of at least 50 days, all the animals were slaughtered with a target LBW of 27 kg. Colon tissue samples were collected for microbiological analysis (stored at -80 ºC during 48 h, then freeze-died), gene expression (RNAlater Invitrogen, Lithuania; stored at -80 ºC), IgA quantification (stored at -20 ºC) and immunohistochemistry examination (fixed by immersion in 10% buffered formalin for one week).

The luminal part of the freeze-died colon samples was scraped and microbial DNA purification was performed with the Ultra-Deep Microbiome Prep kit (Molzym, Life Sciences). Samples of microbial DNA were used as templates for T-RFLP analysis according to Andrés *et al*. (2016), but using MspI as restriction enzyme.

Total RNA was extracted from samples and reversed transcribed using QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). The RNA was used as template for qRT-PCR analysis to evaluate the expression of genes encoding 10 bovine toll-like receptors (TLRs), β-defensin and PGLYRP1 in the epithelium using the gene specific primer pairs relative to β-actin expression.

IgA was quantified in colonic mucosa according to the procedure described by Ahmed *et al*. (2015), using a Genorise ELISA IgA kit (Genorise Scientific, Devon-Berwyn, Pensilvania).

Immunohistochemical labelling of T (polyclonal anti-CD3 antibody; Dako, Milan) and B cells (CD20 antigen, ThermoFisher, Madrid) was carried out with cross sections of the colonic wall samples. Quantification was performed in ten random fields within the lamina propria with final magnification of 40×, using image analysis software (ImageJ v1.6.0_14, National Institutes of Health – NIH, USA).

Data were analyzed using GLM procedure (one-way analysis of variance) of SAS (SAS Institute Inc., Cary, NC), with the milk intake level as the only source of variation. Significance was declared at $P < 0.05$. Those data corresponding to T-RFLP were analyzed by principal component analysis (PCA).

III – Results and discussion

Gastrointestinal mucosa is colonized after birth by pioneer microbes and colonization may be manipulated by early feeding management. Once epimural bacterial population is established, it may deeply impact health, feed efficiency and immune response in later stages of life (Taschuk and Griebel, 2012; Petri *et al*., 2013).

The group that was milk restricted showed a lower gain:feed ratio during the fattening period (0.320 vs. 0.261, P<0.001), being DMI similar for both groups (603 vs. 607 g/day, P>0005). Consequently, RES lambs lasted more time during this period (62 vs. 74 days, P<0.001) to reach the intended body weight of slaughter (27 kg of LBW).

Moreover, the different level of milk intake during the suckling period might explain the clusters observed for the ADL and RES groups when the relative height of terminal restriction fragments (TRF) was analyzed by PCA. An increase of genus *Prevotella* (TRF 99 pb; 0.26% vs 1.17% in the ADL and RES lambs, respectively, P=0.052) and a lower abundance of phylum *Proteobacteria* (TRF 140 pb, 11.17% vs 1.89%, P=0.003; and TRF 152 pb, 2.19% vs. 0.47%, P=0.008) were observed in the colonic mucosa of RES lambs when compared to the ADL group. In accordance with these results, *Prevotella* is a genus commonly found in the large intestine which has been reported in greater abundance in the ruminal liquid of inefficient (high Residual Feed Intake, RFI +) bulls (McCann *et al*., 2014) and within the colon content from steers differing in feed efficiency traits (Myer *et al*., 2015). In addition, *Proteobacteria* is a prevalent phylum in the GIT involved in nutrient digestion that has been previously described in lower relative abundance in the jejunum of low efficient steers (Myer *et al*., 2016).

Nevertheless, the colonic mRNA expression of TLRs and cytokines, number of lymphocytes T and B infiltrated in the lamina propria, and IgA levels were not affected by the different suckling regime (P > 0.005) (Table 1). Thus, the aformentioned changes in the colonic epimural bacterial community between RES and ADL lambs did not seem to promote significant differences in the inmmune parameters analyzed at this level.

Table 1. Toll-like receptors (TLRs) mRNA expression, IgA concentration in colonic mucosa and infiltrating lymphocyte counts in colonic lamina propria of fattening lambs being fed *ad libitum* (ADL) or restricted (RES) during the suckling period

	ADL	RES	RSD	P-value
TLR (ΔCq)				
TLR_1	12.5	12.2	2.03	0.792
TLR_2	20.9	19.2	4.97	0.540
TLR_3	13.1	13.6	2.11	0.681
TLR_4	10.7	10.4	1.73	0.763
TLR_5	21.3	20.8	3.04	0.784
TLR_6	10.1	9.82	2.02	0.789
TLR_7	14.3	14.3	2.68	0.992
TLR_8	10.7	11.1	1.94	0.676
TLR_9	15.5	15.9	1.27	0.522
TLR_{10}	14.9	15.6	2.18	0.562
IgA (pg IgA/µg total protein)	27.6	26.6	2.36	0.394
Lymphocytes (number per field 40×)				
B^+	3.50	3.84	1.272	0.522
T^+	138	159	33.34	0.181

[1] Cq = quantification cycle. ΔCq = Cq (TLR) – Cq (*β*-actin). A smaller ΔCq value represents higher mRNA abundance level.

IV – Conclusions

The results of the present study suggest that early feed restriction during the suckling phase of merino lambs affected feed efficiency and promoted differences in the establishment of the colonic epimural bacterial community, with increased relative abundance of genus *Prevotella* and decreased presence of phylum *Proteobacteria* in milk restricted lambs. However, these modifications could not be associated with differences at local immune response.

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References

Ahmed A.M., Sebastiano S.R., Sweeney T., Hanrahan J.P., Glynn A., Keane O.M., Mukhopadhya A., Thornton K., Good B., 2015. Breed differences in humoral and cellular responses of lambs to experimental infection with the gastrointestinal nematode Teladorsagia circumcincta. In: *Vet Res*, 46.

Andrés S., Bodas R., Tejido M.L., Giráldez F.J., Valdés C., López S., 2016. Effects of the inclusion of flaxseed and quercetin in the diet of fattening lambs on ruminal microbiota, in vitro fermentation and biohydrogenation of fatty acids. In: *J Agri Sci*, 154, p. 542-552.

Animal Task Force, 2013. Research & innovation for a sustainable livestock sector in Europe. In: *An Animal Task Force White Paper*, p. 1-24.

McCann J.C., Wiley L.M., Forbes T.D., Rouquette Jr F.M., Tedeschi L.O., 2014. Relationship between the Rumen Microbiome and Residual Feed Intake-Efficiency of Brahman Bulls Stocked on Bermudagrass Pastures. In: *PLoS ONE*, 9.

Myer P.R., Wells J.E., Smith T.P.L., Kuehn L.A., Freetly H.C., 2015. Microbial community profiles of the colon from steers differing in feed efficiency. In: *SpringerPlus,* 4, p. 454.

Myer P.R., Wells J.E., Smith T.P.L., Kuehn L.A., Freetly H.C., 2016. Microbial community profiles of the jejunum from steers differing in feed efficiency. In: *J Anim Sci*, 94, p. 327-338.

Petri R.M., Schwaiger T., Penner G.B., Beauchemin K.A., Forster R.J., McKinnon J.J. and McAllister T.A., 2013. Changes in the Rumen Epimural Bacterial Diversity of Beef Cattle as Affected by Diet and Induced Ruminal Acidosis. In*: Appl Environ Microbiol*, 79, p. 3744-3755.

Taschuk R., Griebel P.J., 2012. Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. In: *Anim Health Res*, 13, p. 129-141.