Effect of dietary addition of EPA, DPA and DHA on rumen bacterial community in cows and ewes. An *in vitro* approach

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Take home message Dietary addition of EPA, DPA, and DHA altered the abundance of several bacterial genera, such as *Prevotella*, *Hallella*, *Paraprevotella* and *Succiniclasticum*, that might participate in rumen biohydrogenation.

Introduction The role of marine lipids as modulators of rumen biohydrogenation (BH) of unsaturated fatty acids (FA) is likely to be due to the impact of their n-3 polyunsaturated FA on the microbiota (Toral *et al.*, 2017). However, the effect of individual FA, namely 20:5 n-3 (EPA), 22:5 n-3 (DPA), and 22:6 n-3 (DHA) has rarely been studied and it is still uncertain which rumen bacteria are involved in the BH process. Moreover, despite interspecies differences in the rumen bacterial composition (Toral *et al.*, 2016), we are not aware of any reports comparing the effects of these FA in cows and sheep. Therefore, rumen inocula from these species were used to examine *in vitro* the effect of EPA, DPA and DHA on the bacterial community. A concomitant study had analysed their influence on ruminal fermentation and BH (Toral *et al.*, 2017).

Materials & methods Batch cultures of rumen microorganisms were conducted using inocula from 2 ruminant species (*i.e.*, 2 cannulated cows and 2 cannulated ewes fed a TMR with a forage:concentrate ratio 50:50) and 4 treatments (control –the TMR without supplementation–, and the TMR plus 2% DM of EPA, DPA or DHA). Incubations lasted for 24 h and were repeated on 3 consecutive days. DNA was extracted from freeze dried samples and bacterial 16S rRNA (V1-V2) amplicon was analysed by Ion Torrent PGM next generation sequencing (de la Fuente *et al.*, 2014). The effect of animal species and treatments on the taxa's relative abundance was analysed by ANOVA, with the MIXED procedure of the SAS 9.4.

Results & discussion Only effects on bacteria that were previously suggested to be somehow related to rumen BH are reported here. Most changes were comparable in both species, but some groups shifted with EPA and/or DHA only in cattle (*e.g.*, increases in *Hallella*, *Ruminococcus* and *Ruminobacter*) or only in sheep (increments of *Paraprevotella* and decreases in *Oscillibacter*). *Prevotella* abundance was reduced by all treatments, especially EPA and DHA, in the two animal species (Figure 1A). This genus has been related to 18:0 formation (Huws *et al.*, 2011), which is consistent with observations by Toral *et al.* (2017). Abundances of *Hallella* in cattle and *Paraprevotella* in sheep increased similarly with EPA and DHA (Figure 1B). These microorganisms form succinate, a precursor of propionate for *Succiniclasticum* spp., whose abundance raised with all treatments in cattle and with DHA in sheep (Figure 1C). A putative association between propionate formation and a shift in BH pathways was previously speculated in Toral *et al.* (2017) and suggest that bacteria related to propionate metabolism might play a part in those BH routes.

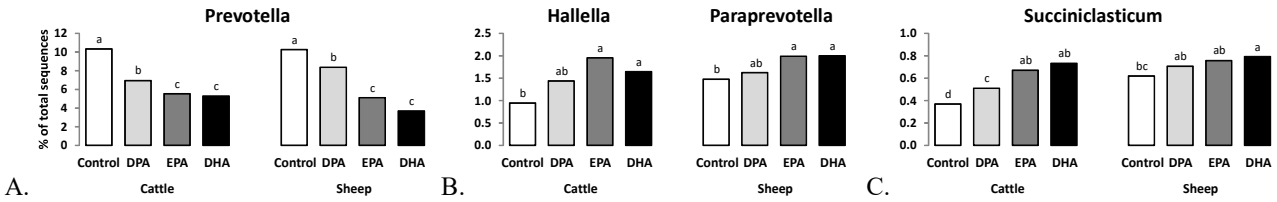

Figure 1 Relative abundances of *Prevotella* (A), *Hallella* and *Paraprevotella* (B), and *Succiniclasticum* (C) in ruminal digesta of cattle and sheep after 24 h-incubations without FA supplementation (Control) or with DPA, EPA or DHA.

Conclusion Dietary addition of 2% DM of EPA, DPA and DHA in *in vitro* batch cultures alter some bacterial genera potentially involved in lipid metabolism, such as *Prevotella*, *Hallella*, *Paraprevotella* and *Succiniclasticum*. Most changes were comparable in sheep and cows, but there were also variations exclusive to each ruminant species. The effect of DPA was less pronounced than that of EPA or DHA.

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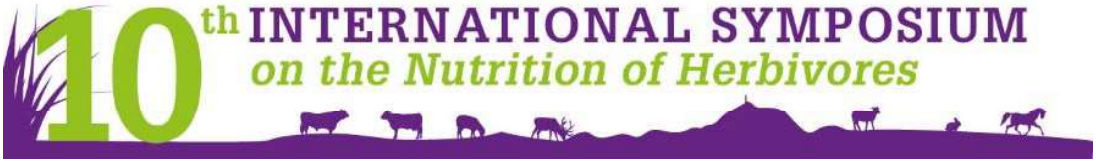

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TAKE-HOME MESSAGE

Dietary addition of **EPA, DPA, and DHA** altered the **abundance** of several bacterial genera, such as *Prevotella*, *Hallella*, *Paraprevotella* and *Succiniclasticum*, that might participate in rumen **biohydrogenation**.

INTRODUCTION

The role of **marine lipids** as modulators of rumen biohydrogenation (BH) of unsaturated fatty acids (FA) is likely to be due to the **impact of their n-3 polyunsaturated FA** on the **microbiota**. However, the effect of individual FA, namely **20:5 n-3 (EPA), 22:5 n-3 (DPA), and 22:6 n-3 (DHA)**, has rarely been studied and it is still uncertain which rumen bacteria are involved in BH. Despite **interspecies differences** in the rumen bacterial composition (Toral *et al.*, 2016), we are not aware of any reports comparing the effects of these FA in cows and sheep.

Therefore, rumen inocula from **cattle and ewes** were used to examine *in vitro* the effect of **EPA, DPA and DHA** on the bacterial community. A concomitant study had analysed their influence on ruminal fermentation and BH (Toral *et al.*, 2017).

MATERIAL AND METHODS

Batch cultures of rumen microorganisms

Substrate - TMR with a forage:concentrate ratio 50:50

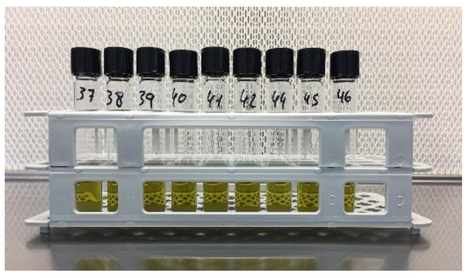
Inoculum - Rumen fluid from cannulated animals (3 runs on different days)

Incubation time - 24h

Species - Cattle (2 cows) and sheep (2 ewes)

Treatments

- TMR without supplementation (Control)
- TMR + 2% DM of 20:5 n-3 (EPA)
- TMR + 2% DM of 22:5 n-3 (DPA)
- TMR + 2% DM of 22:6 n-3 (DHA)



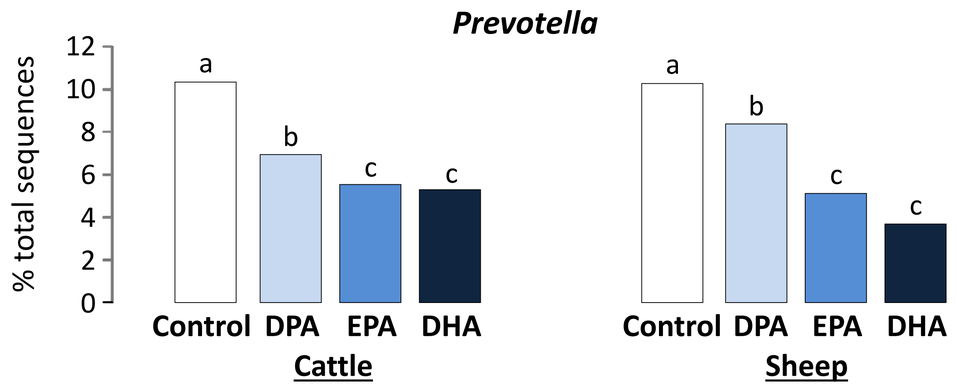
DNA extraction and sequencing

Bacterial 16S rRNA (V1-V2)

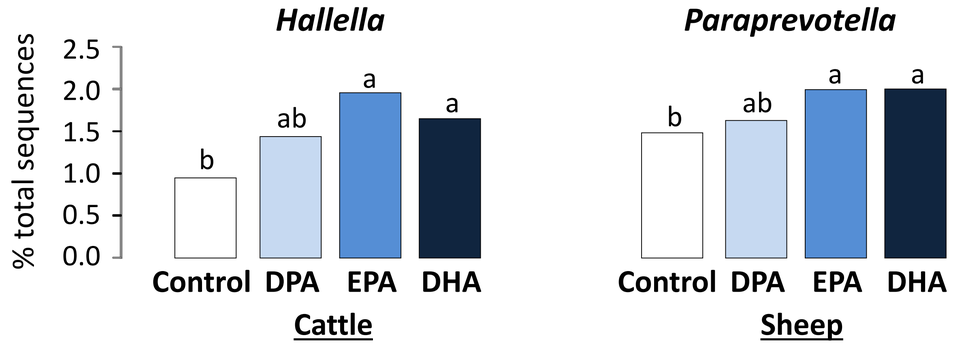
Ion Torrent PGM next generation sequencing

RESULTS

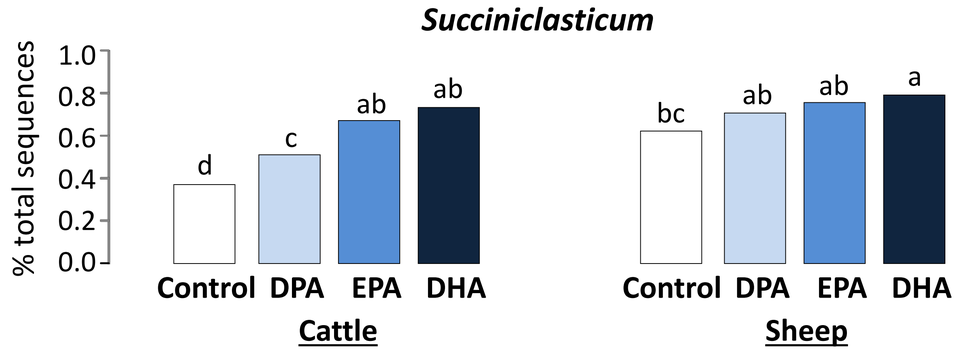
Only effects on bacteria previously suggested to be related to rumen BH are reported. Most changes were comparable in both species, but some groups shifted with EPA and/or DHA only in cattle (*e.g.*, *Hallella*, *Ruminococcus* and *Ruminobacter*) or only in sheep (*e.g.*, *Paraprevotella* and *Oscillibacter*).



Prevotella abundance was reduced by all treatments, especially EPA and DHA, in both species. This genus has been related to 18:0 formation (Huws *et al.*, 2011).



Abundances of *Hallella* in cattle and *Paraprevotella* in sheep increased similarly with EPA and DHA.



Hallella and *Paraprevotella* form succinate, a precursor of propionate for *Succiniclasticum* spp., whose abundance raised with all treatments in cattle and with DHA in sheep.

An association between propionate formation and a shift in BH pathways was previously speculated (Toral *et al.* 2017), suggesting that bacteria related to propionate metabolism might play a part in those BH routes.

CONCLUSION

Dietary addition of 2% DM of **EPA, DPA and DHA** in *in vitro* batch cultures alter some **bacterial genera potentially involved in lipid metabolism** (e.g., *Prevotella*, *Hallella*, *Paraprevotella* and *Succiniclasticum*). Most changes were **comparable in sheep and cows**, but there were also variations exclusive to each ruminant species. The effect of **DPA** was **less pronounced** than that of EPA or DHA.

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