Global Methane Hub

ENTERIC FERMENTATION
R&D ACCELERATOR

STATE OF SCIENCE

Balance of H_2-forming and H_2-consuming fermentations differs by 25%

Prepared By: Karen Beauchemin and Rolf Thauer

The amount of H_2 formed and consumed in the rumen can be estimated from the amount of methane formed: $4\ H_2 + CO_2 = CH_4 + 2\ H_2O$. This is based on the assumption that acetogenic bacteria synthesizing acetate from $2\ CO_2$ and H_2 ($4\ H_2 + 2\ CO_2 = CH_3COOH + 2\ H_2O$) are not significantly involved in the rumen fermentation. Based on that assumption attempts have been made to estimate the amount of H_2 formed as intermediate from the amount of acetate, ethanol, propionate, butyrate and caproate generated. Per mol acetate formed from hexoses 2 mol H_2 should be formed. Butyrate is formally synthesized from 2 acetate and 2 H_2, propionate from acetate, CO_2 and 3 H_2, caproate from 3 acetate and 4 H_2 and ethanol from acetate and 2 H_2. However, the amount of intermediate H_2 estimated via this calculation was found to be significantly higher (at least 25%) than that calculated via methane (Guyader et al. 2017). The H-imbalance is most easily explained if the assumption

made in the calculations is wrong that the rumen fermentation does not involve CO_2 reduction with H_2 to acetate. Then the formation of acetate would be both a source and a sink for electrons and therefore no longer an indicator for H_2 formation. It should be noted that these H-balance studies have been done mainly in vitro, namely using the Rumen Simulation Technique (RUSITEC), as it is presently not yet possible to measure volatile fatty acid production rates in the rumen accurately in vivo. Even In fistulated ruminants using isotopes it is difficult to quantitatively account for the many interconversions and absorption of the volatile fatty acids.

When methanogenesis in dairy cows is inhibited by 30% using 3-nitrooxypropanol (3-NOP), then about 10 mol of methane, equivalent to 80 mol electrons, are lost less per day to the atmosphere. About 10% of these electrons show up in exhaled H_2, whose steady state concentration in the rumen increases drastically, showing the importance of CH_4 formation as a H_2 sink in the rumen. However, it is not known where the rest of the electrons end up (Hristov et al., 2015; Lopes et al., 2016). As eluted above, the total synthesis of acetate from 2 CO_2 is one possible explanation. In addition, the slowdown in the hydrolysis of the polysaccharides to mono- and disaccharides as the indirect result of the increase of the H_2 concentration has to be considered: less carbohydrates fermented, less H_2 formed. However, there is no convincing experimental evidence for either of these two possible explanations.

CO_2 reduction to formate and the reduction of polyunsaturated fatty acids to their more saturated counterparts have been considered as sinks of electrons responsible for the H_2 imbalance but have been disregarded as not being of quantitative importance (Guyader et al., 2017). Another possibly missed electron sink frequently discussed is the biosynthesis of microbial cells from carbohydrates. However, only up to 10% of the carbohydrate carbon ends up in microbial cells and the redox state of cell carbon is near zero, which is the oxidation state of carbon in the carbohydrates. Therefore, cell formation from carbohydrates should have only a minor effect on the H balance calculated from the fermentation products.

Butyrate-, propionate-, caproate- and ethanol formation are described above as pathways of H_2 utilization, but this is not strictly true. Instead, butyrate-, propionate- and ethanol formation is an alternative to H_2 formation since these pathways are accepting electrons generated during sugar oxidation to pyruvate and acetyl-CoA + CO_2 (Janssen 2010). Similarly, it is not acetate but acetyl-CoA that is reduced to butyrate, propionate and ethanol. When added, only trace amounts of label from 13C-acetate were incorporated into butyrate and propionate in rumen samples (Le Van et al., 1998).

With the exhaled methane, ruminants lose about 6 % of the energy contained in the feed. Dependent on the feed, the loss can be as low as 3% and as high as 14% (Johnson and Johnson, 1995). The general assumption therefore is that when methanogenesis is inhibited, this should be of benefit for the ruminant. However, a convincing increase in feed efficiency in the presence of anti-methanogenics has not yet been observed, which - like the 25% H-imbalance - is not understood. Is there a link between the two? These questions matter because farmers will probably be motivated to use anti-methanogenics as feed additives only if there is a benefit.

LITERATURE CITED

Guyader, J., Ungerfeld, E.M., and Beauchemin, K.A. (2017). Redirection of metabolic hydrogen byinhibiting methanogenesis in the rumen simulation technique (RUSITEC). Front Microbiol 8. 10.3389/fmicb.2017.00393.

Hristov, A.N., Oh, J., Giallongo, F., Frederick, T.W., Harper, M.T., Weeks, H.L. et al. (2015). An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proceedings of the National Academy of Sciences of the United States of America 112, 10663-10668. 10.1073/pnas.1504124112.

Janssen, P.H. (2010). Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim Feed Sci Tech 160, 1-22. 10.1016/j.anifeedsci.2010.07.002.

Johnson, K.A., and Johnson, D.E. (1995). Methane emissions from cattle. J Anim Sci 73, 2483-2492.

Le Van, T.D., Robinson, J.A., Ralph, J., Greening, R.C., Smolenski, W.J., Leedle, J.A.Z. et al. (1998). Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and Acetitomaculum ruminis. Appl Environ Microb 64, 3429-3436.

Leng, R.A. (2014). Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. Anim Prod Sci 54, 519-543. 10.1071/An13381

Lopes, J.C., de Matos, L.F., Harper, M.T., Giallongo, F., Oh, J., Gruen, D. et al. (2016). Effect of 3-nitrooxypropanol on methane and hydrogen emissions, methane isotopic signature, and ruminal fermentation in dairy cows. J Dairy Sci 99, 5335-5344. 10.3168/jds.2015-10832