Role of nitric oxide production in dairy cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

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Nitric oxide (NO) is a crucial mediator in host defense and is one of the major killing mechanisms within macrophages. Its induction is highly affected by the types of cytokines and the infectious agents present. In the current study, NO production was evaluated after *in vitro* infection of unfractionated peripheral blood mononuclear cells (PBMCs) with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) after 8 hr, 3 and 6 days of culture for cows in different stages of disease. In addition, the effects of *in vitro* exposure to inhibitory cytokines such as interleukin-10 (IL-10) and transforming growth factor β (TGF-β) as well as the pro-inflammatory cytokine IFN-γ were correlated with the level of NO production. Nitric oxide production was consistently higher in cell cultures from subclinically infected animals at all time points. An upregulation of NO production was demonstrated in unfractionated cell cultures from healthy control cows after exposure to MAP infection as compared to noninfected cell cultures. A similar increase in NO due to the addition of MAP to cell cultures was also noted for clinically infected cows. NO level among subclinically infected cattle was greater at all time points tested and was further boosted with the combination of both *in vitro* MAP infection and IFN-γ stimulation. Finally, the *in vitro* exposure to inhibitory cytokines such as IL-10 and TGF-β prior to MAP infection or LPS stimulation resulted in the downregulation of this inflammatory mediator (NO) in all experimental groups at all time points. In summary, a higher level of NO production was associated with cows in the subclinical stage of MAP infection.