Presentation PSIII-38

Butyric acid and derivatives: In vitro anti-inflammatory effects tested in porcine alveolar macrophages

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Background

- Butyric acid and its derivatives have beneficial effects when included in animal feed and they are currently under investigation for its effects on host cells *in vitro.*
- Butyrate may be an effective feed additive because it modulates immune response of host cells *in vitro* (Weber and Kerr, 2006; Chen and Vitetta, 2018).
- Porcine alveolar macrophages (PAMs) can be isolated from weanling piglets by methods described by Liu et al., (2013) and cultured with a lipopolysaccharide challenge to induce inflammatory response.
- Butyric acid (**Fig. 1**) and its derivatives, sodium butyrate (**Fig. 2**), monobutyrin (**Fig. 3**), and tributyrin (**Fig. 4**) are different compounds which may deliver butyrate *in vivo.*

Fig. 1

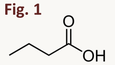


Fig. 2

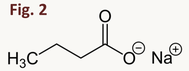


Fig. 3

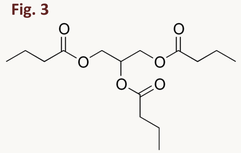
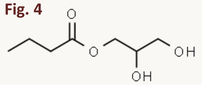


Fig. 4



Objective

To examine the anti-inflammatory effects of butyric acid, sodium butyrate, monobutyrin and tributyrin using porcine alveolar macrophages (PAMs).

Materials and methods

- Bronchial lavage with ~100 mL ice-cold PBS from 6 healthy weaned piglets was used to isolate porcine alveolar macrophages (PAMs).
- PAMs were seeded at 10^6 cells/mL and cultivated overnight.
- 2×5 factorial experimental design, n=12:
  - Factor 1: 5 levels of butyric acid or derivatives
    - Doses: Butyric acid, tributyrin—0, 0.5, 1, 2, 4 mM; Monobutyrin, sodium butyrate—0, 1, 2, 4, 8 mM.
  - Factor 2: with or without 1 µg/mL lipopolysaccharide (LPS) challenge

- **MTT assay: Cytotoxicity of treatments**
  Colorimetric assay using 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) was performed to determine cell viability expressed as percent of control.
- **Anti-inflammatory effects**
  - Supernatants were collected after 24h with treatment.
  - Supernatants were analyzed by enzyme-linked immunosorbent assay (ELISA) for cellular secretion of tumor-necrosis factor alpha (TNF-α).

Results

Figure 5. TNF-α secretion by LPS-challenged PAMs

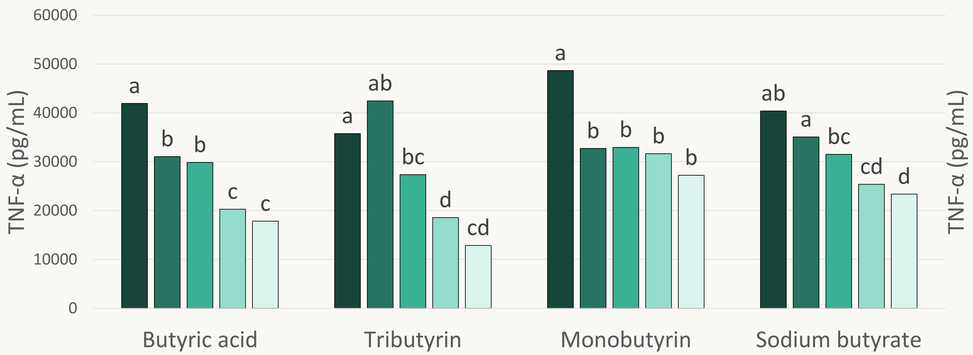
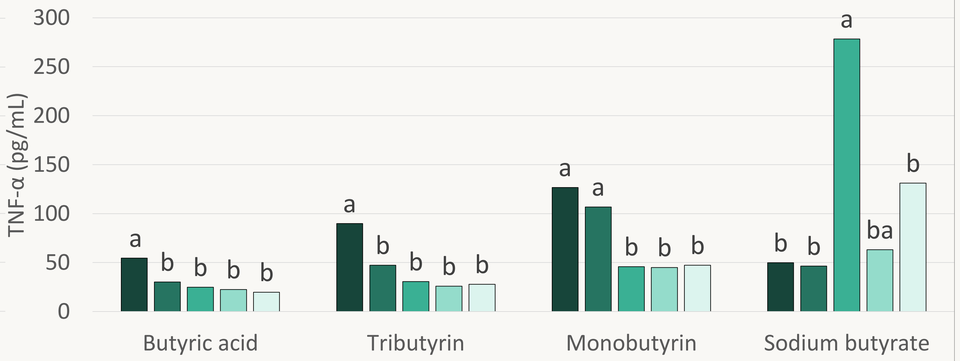


Figure 6. TNF-α secretion by non-challenged PAMs



0 mM ■ BA/TB: 0.5 mM, SB/MB: 1 mM ■ BA/TB: 1 mM, SB/MB: 2 mM ■ BA/TB: 2 mM, SB/MB: 4 mM ■ BA/TB: 4 mM, SB/MB: 8 mM

- **MTT assay (data not shown)**
  - Cells at all tested doses were considered viable (≥76% of sham control)
  - Sodium butyrate at 2 and 4 mM dose exhibited ($P < 0.01$) a stimulatory effect on cell proliferation.
- **Anti-inflammatory effects**
  - LPS challenge remarkably stimulated ($P < 0.0001$) TNF-α secretion from PAMs.
  - All compounds reduced TNF-α secretion dose-dependently ($P<0.001$) (**Figure 5**).
  - Non-challenged PAMs secreted less TNF-α compared with control for all compounds except sodium butyrate, which tended to increase TNF-α secretion at 2 mM ($P = 0.056$) (**Figure 6**).

Conclusions

- **Butyric acid, tributyrin, and monobutyrin reduce TNF-α secretion in non-challenged cells.**
- **Sodium butyrate may induce TNF-α secretion at higher doses.**
- **Butyric acid, tributyrin, monobutyrin, and sodium butyrate dose-dependently reduce the secretion of TNF-α by Porcine Alveolar Macrophages challenged with lipopolysaccharide (1 µg/mL).**

References

1. Chen, J., and L. Vitetta. 2018. Inflammation-modulating effect of butyrate in the prevention of colon cancer by dietary fiber. Clinical Colorectal Cancer. 17:e541–e544. doi:10.1016/j.clcc.2018.05.001.
2. Weber, T. E., and B. J. Kerr. 2006. Butyrate differentially regulates cytokines and proliferation in porcine peripheral blood mononuclear cells. Veterinary Immunology and Immunopathology. 113:139–147. doi:10.1016/j.vetimm.2006.04.006.
3. Liu Y., Song, M., Che, T. M., Bravo, D., Pettigrew J., E. 2012. Anti-inflammatory effects of several plant extracts on porcine alveolar macrophages in vitro. J Anim Sci. 90(8):2774-2783.

Acknowledgements