Betafin® natural betaine

TECHNICAL REPORT

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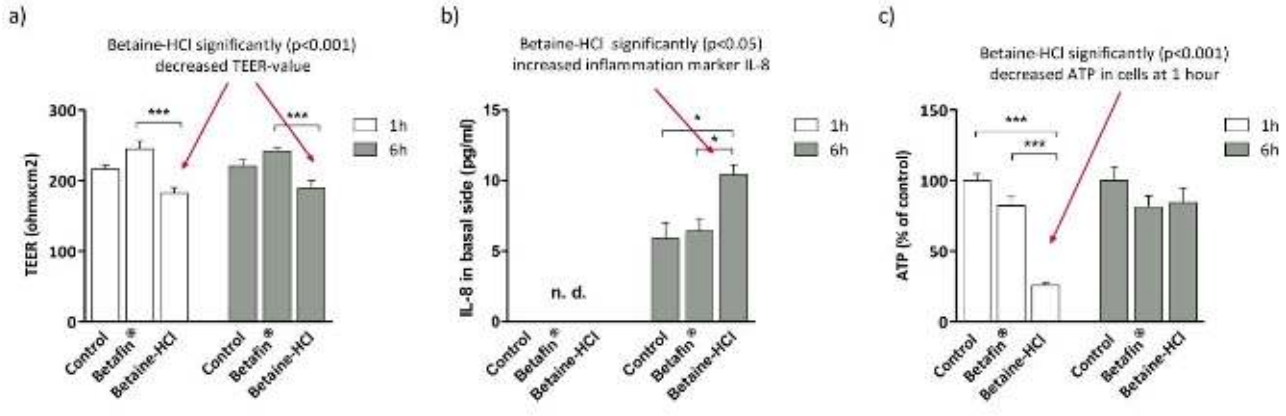
Betaine-HCl negatively influenced the gut barrier and ATP content of cells compared to Betafin® S1 natural betaine in an in vitro intestinal cell model

Trial site: DuPont Nutrition and Health, Kantvik Active Nutrition, Finland

Natural betaine has two roles in nutrition. As an osmoregulator it can protect cell enzyme systems and membranes from ionic inactivation during stress[1,2,4,5]. As a methyl donor via transmethylation, it is more effective than other potential methyl group donors such as methionine and choline [1,2,4,8]. Natural betaine benefits parameters such as bodyweight gain[1,2,8], feed utilisation and costs [1,2,8,9], carcass lean deposition [1,2,11] and litter size [10,12], with effects magnified at times of production stress (e.g. heat stress, coccidiosis challenge) [3,4,6,7].

- Betaine hydrochloride (betaine-HCl) significantly (P<0.05) reduced transepithelial electrical resistance (TEER) values compared to Betafin® S1 natural betaine in an intestinal epithelial cell (IEC) model. Weak tight junctions between cells can lead to movement of antigens across the gut barrier to provoke a damaging and energetically costly inflammatory response.
- Betaine-HCl increased cell inflammation compared to both the control and Betafin® S1 natural betaine groups, as indicated by significantly (P<0.05) higher cytokine IL-8 production. Cytokine IL-8 activates an inflammatory response in immune cells.
- Betaine-HCl significantly (P<0.05) decreased ATP content of the cells compared to the control and Betafin® S1 natural betaine. ATP is crucial to sustain metabolic functions within cells.

Results: Effect of *in vitro* digested feed samples supplemented with either Betafin® S1 natural betaine (96% betaine) or betaine-HCl (71% betaine) at equal betaine content (2 kg/tonne of feed) on (a) tight junction strength (TEER value) (b) cytokine IL-8 release and (c) ATP content of cells in an *in vitro* intestinal epithelial cell model



n.d. = under detection limit.

Transepithelial electrical resistance (TEER) measurement

TEER measures the integrity of the tight junctions between cells as resistance units (ohm × cm^2). The higher the TEER value, the tighter are the cell junctions. A decrease in TEER value indicates that there is a possibility of the passage of luminal antigens through the tight junctions towards the basolateral side or the "inner body". This movement of luminal antigens may cause an inflammatory response. The results show that betaine-HCl induced a significant drop in the TEER value compared to Betafin® S1 natural betaine after 1 h and this effect still persisted at 6 h.

Cytokine IL-8

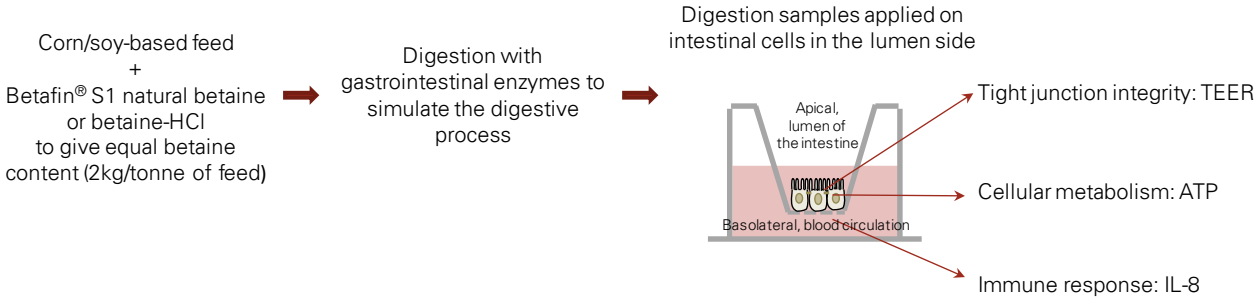
IL-8 (interleukin-8) is a cytokine with a major function to attract neutrophils (a class of white blood cell) to the site of inflammation and to activate inflammatory responses in immune cells. IL-8 production may be induced in epithelial cells by infection or cellular stress. Betaine-HCl induced an increase in the amount of IL-8 after 6 hrs which was significantly higher than both the control group and the Betafin® S1 natural betaine-supplemented groups. This suggests that betaine-HCl triggers an inflammatory response in the epithelium *in vivo* which might subsequently lead to a decrease in tight junction integrity and leakage of apical "luminal" contents to basolateral side or "inner body". These results are therefore consistent with the changes in TEER values seen.

ATP content of cells

ATP content determines the functional integrity of the cells as ATP is required for cells to stay alive and to sustain basic metabolic functions. Any form of cell injury causes a rapid decrease in the ATP content of the cells, which would then negatively affect the metabolism of the cells. Betaine-HCl caused a significant drop in the amount of ATP in the epithelial cells both compared to the control and Betafin® S1 natural betaine after 1 h. This effect was not seen at 6 h, which indicates that the epithelial cells were able to repair themselves and restore the ATP production after betaine-HCl treatment. However, there may be an 'energetic cost' to this restoration process in an *in vivo* situation.

Design:

Intestinal epithelial cells (IECs) (Caco-2) differentiated for 21 days in asymmetric conditions were used to study the effect of *in vitro* digested feeds supplemented with betaine at 2kg/tonne of feed, either from Betafin® S1 natural betaine (96% betaine) or betaine-HCl (71% betaine) on: 1) the integrity of the tight junctions, 2) cytokine IL-8 release and 3) ATP content in cells (see figure below). A corn/soy-based diet was either unsupplemented (control) or supplemented with one of the two sources of betaine. The three diets were exposed to *in vitro* digestion to mimic the chicken proximal digestive tract via use of HCl, pepsin and pancreatin after which the pre-digested feed was applied to the cells on the lumen (apical) side. One replicate of the control treatment digestion was made and two replicates of digestion were made for the betaine-supplemented feeds. After the *in vitro* digestion, the added digestive enzymes were inactivated by heating and the soluble fractions were diluted in 1:1 in bicarbonate- and serum-free cell culture medium and applied in the compartment modeling the intestinal lumen. Transepithelial electrical resistance (TEER), ATP content (ATPlite, Perkin Elmer) and the basolateral cytokine IL-8 (Multiplex ELISA, Aushon Biosystems) were measured at 1 and 6 hours after adding the test solutions to the cells.



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Further supporting references are available on request from Danisco Animal Nutrition

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