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Assessing the capacity of mustard by-product to replace medicinal zinc oxide in co-culture cells

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Aim of the study

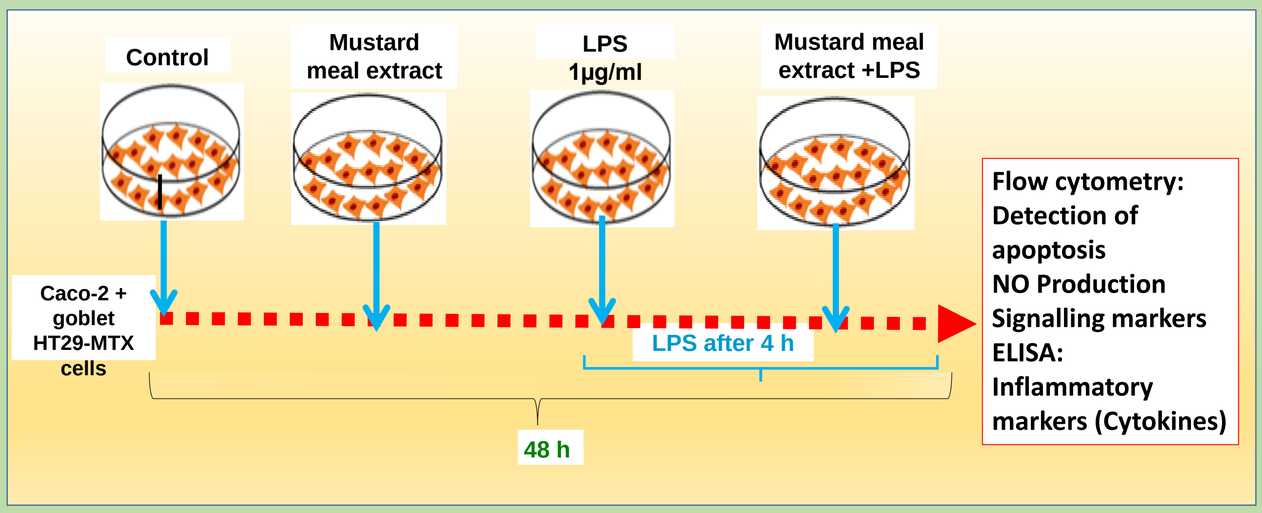
The present study investigated in vitro the capacity of a mustard meal extract to counteract the pro-inflammatory effect induced by the E. coli toxin LPS in the co-culture Caco-2 and HT-29 MTX cells used as a cellular model to mimic the intestinal barrier epithelium.

Introduction

- The banning of antibiotics (2006) as in feed growing promoters and of medicinal zinc oxide (2023) in Europe will have a significant impact on the livestock sector, especially on pigs, a very sensitive category to infections with *Escherichia coli*, *Salmonella*, *Rotavirus* during and after the weaning period. This situation has given the opportunity to animal nutrition research to find alternatives: biologically active compounds efficient in maintaining the animal health.
- Mustard seed meal (MSM) obtained after oil extraction could be a alternative source of bioactive compounds being rich in polyphenols, polyunsaturated fatty acids (PUFA omega-9, -6, -3), minerals (Cu, Zn, Mn, Fe), carbohydrates and others, known for their antimicrobial and immune stimulating activity.

Material and method

- Study was performed in a complex intestinal co-culture cellular model including a monolayer enterocyte Caco-2 and goblet HT29-MTX cells challenge with *Escherichia coli*- LPS as one of the most common intestinal pathogens. Mustard meal extract was obtained with methanol 80%. Apoptosis and nitric oxide production as well as signalling molecules (MAKs/ PTK) involved in their mechanism was determined by flow cytometry while pro-inflammatory markers were determined by ELISA.



Results and discussion

Bioactive compounds in mustard meal

- The results showed that mustard meal are rich in polyphenols (988.6 mg GAE/100g), of which the highest concentration being found for catechin (55.95 mg/100g), one of the most powerful antioxidants, polyunsaturated fatty acids, the highest level being recorded for cis-oleic acid (46.25%), followed by cis-linoleic acid (34.04g FAME/100gTotal FAME) and linolenic acid (7.32 FAME/100gTotal FAME). Mustard meal contain mineral elements, essential for the development of a rapid and adequate immune response, such as iron (107.82 ppm), copper (3.44 ppm), manganese (30.42 ppm) and zinc (56.34 ppm). Carbohydrates (glucose, fructose, sucrose) and organic acids (succinic, malic, citric) essential for modulating the intestinal microflora were also determined in high concentration in mustard meal.

Results and disscusion

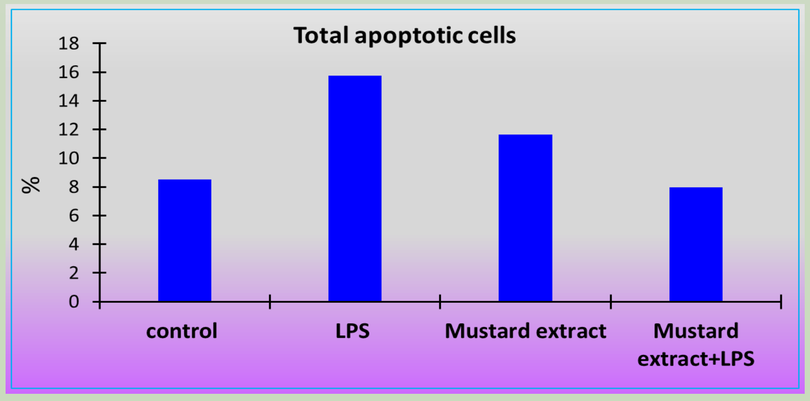


Figure 1. Effect of LPS and mustard meal extract on cell apoptosis.
The exposure of intestinal cells E.coli-LPS increased the level of apoptosis. By contrast the presence of mustard extract reduced exacerbated LPS effect.

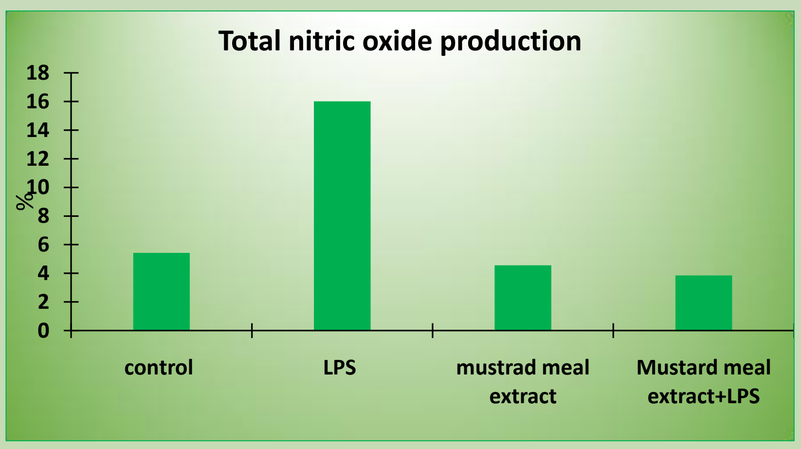


Figure 2. Effect of LPS and mustard meal extract on nitric oxide production.
NO production was significantly induced by LPS alone while in the cells treated with mustard extract NO production recovered the control level.

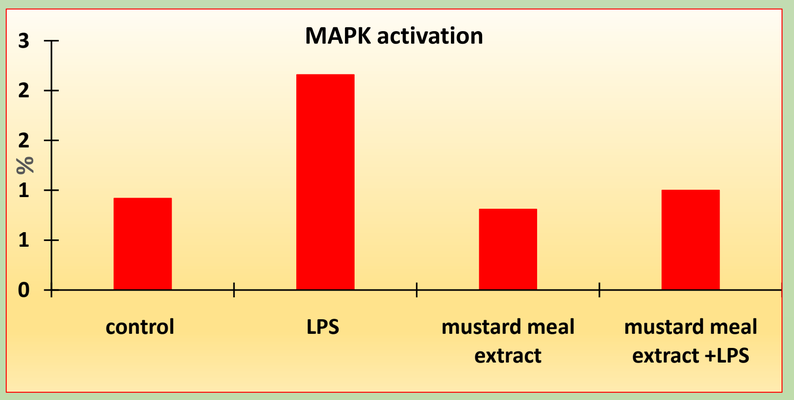


Figure 3. Effect of LPS and mustard meal extract on signalling MAPK activation.
As expected LPS strongly activated MAPK involved in inflammatory pathway. Mustard meal extract had the potential to counteract the effect of LPS.

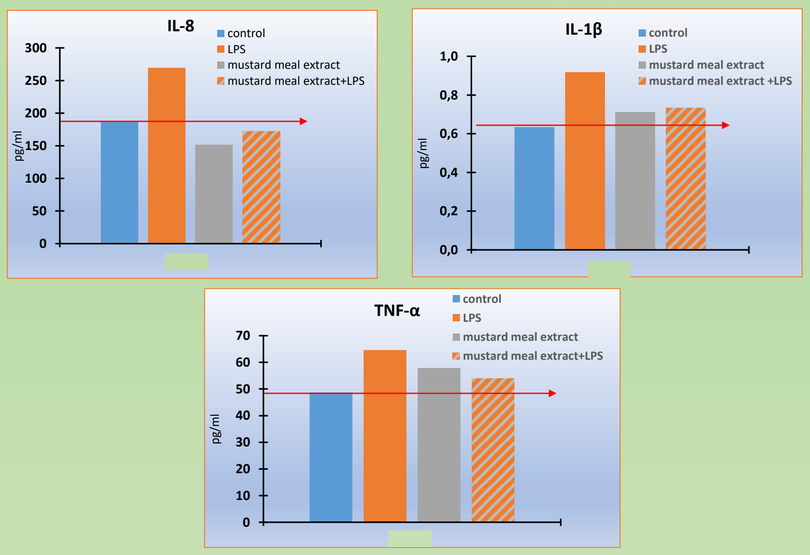


Figure 4. Effect of LPS and mustard meal on pro-inflammatory cytokines.
Treatment of cells with E.coli –LPS strongly increased the concentration of pro-inflammatory cytokines whereas mustard meal extract was able to decreased the cytokine response toward the control level

Conclusions

- Mustard meal extract was able to counteract the harmful effect produced by LPS on cell apoptosis, nitric oxide production and pro-inflammatory cytokines.
- Further in vivo studies are necessary to validate the *in vitro* results.

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