Enhancement of the cellular antioxidant system and tight junctions by a plant sterols food supplement in a co-culture model of intestinal inflammation

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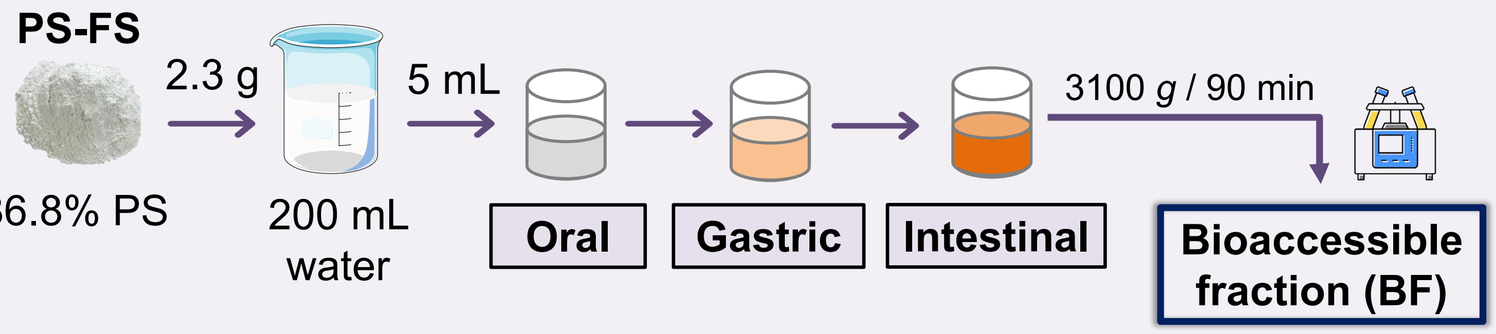
INTRODUCTION & AIM

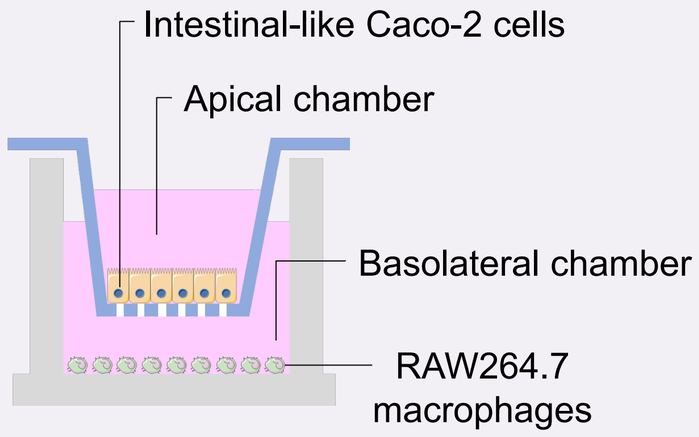
Previous studies indicate that a plant sterol-based food supplement (PS-FS) reduces markers of intestinal inflammation in both cell co-culture and animal models.[1,2] It has been shown to decrease the secretion of pro-inflammatory cytokines, mitigate oxidative and nitrosative stress, alleviate clinical symptoms, restore the histological structure of colonic tissue and increases levels of the tight junction protein occludin. These effects are linked to the suppression of the NF-κB p65-COX-2-PGE_2 signaling pathway. However, it remains unclear whether PS-FS may also impact additional dysregulated pathways in intestinal inflammation. This study, therefore, aims to investigate the effects of PS-FS on the expression of genes associated with the cellular antioxidant defense system and tight junction proteins.

METHODOLOGY

1 *In vitro* gastrointestinal digestion (INFOGEST 2.0)[3]



2 Co-culture model of intestinal inflammation[1]



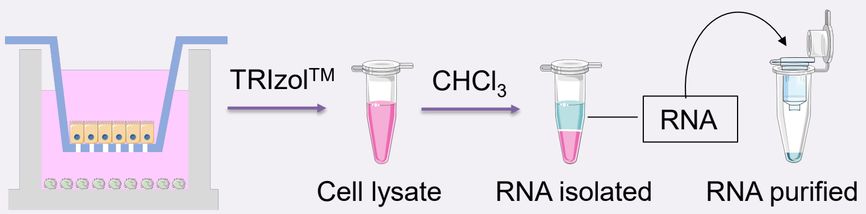
90 min (Apical): BF (1/20, v/v); Budesonide (1µM); BF + Budesonide

24 h (Basolateral): LPS (1 µg/mL)

Combination index (CI)
1 = Additive
> 1 = Synergism
< 1 = Antagonism

3 Gene expression analysis[4]

RNA extraction from Caco-2 cells



RT-qPCR

TaqMan™ Reverse Transcription: 25ºC 10 min → 48ºC 30 min → 95ºC 5 min

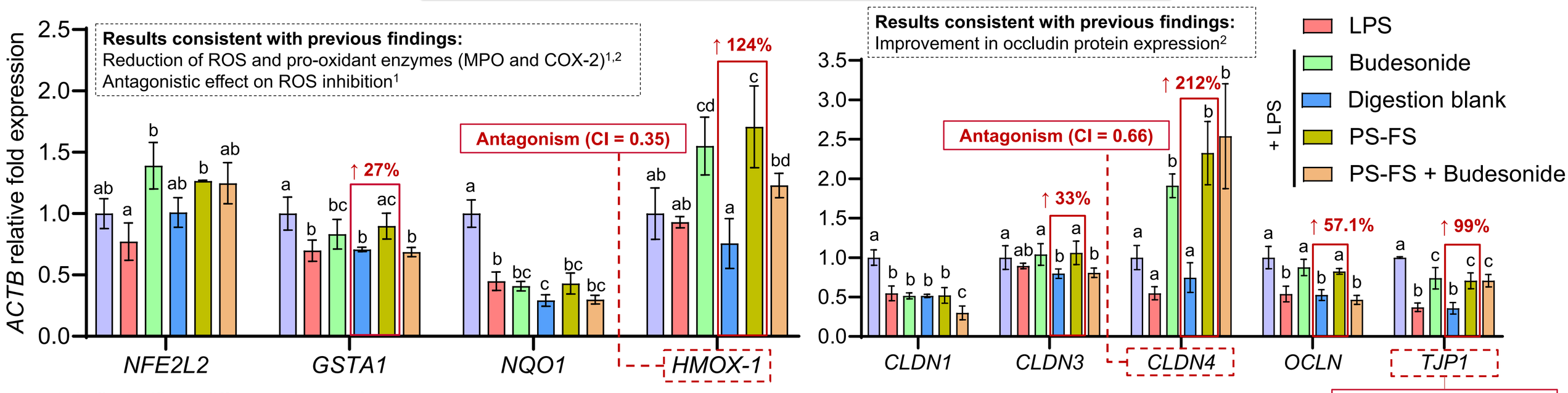
PowerUp™ SYBR™ Green: 95ºC 5 min → 40 cycles [95ºC 10 s → 60ºC 30 s → 72ºC 15 s]

Genes evaluated

Cellular antioxidant system	Tight junctions
Nrf2 (*NFE2L2*)	Claudins-1, -3, and -4 (*CLDN1, 3*, and *4*)
Glutathione S-transferase A1 (*GSTA1*)	Occludin (*OCLN*)
NAD(P)H quinone dehydrogenase 1 (*NQO1*)	Zonula occludens-1 (*TJP1*)
Heme oxygenase 1 (*HMOX-1*)	

Scan the QR code to access the primer sequences used and their efficiency

RESULTS & DISCUSSION



Results consistent with previous findings:
Reduction of ROS and pro-oxidant enzymes (MPO and COX-2)[1,2]
Antagonistic effect on ROS inhibition[1]

Results consistent with previous findings:
Improvement in occludin protein expression[2]

Statistically significant differences ($p < 0.05$) between conditions for the same gene are indicated by different letters (a-d)

CONCLUSION

PS-FS demonstrated potential to enhance the expression of genes involved in antioxidant responses (*GSTA1* and *HMOX-1*) and intestinal barrier maintenance (*CLDN3*, *CLDN4*, *OCLN* and *TJP1*), suggesting that it could be a promising dietary intervention for inflammatory bowel disease. However, co-treatment with budesonide revealed antagonistic effects, highlighting the importance of further research on this interaction and the potential need to avoid their combined use.

REFERENCES

[1]Faubel et al. (2024). *Food & Function*, 15, 6502-6511. [2]Makran et al. (2024). *Food Science & Human Wellness* (https://doi.org/10.26599/FSHW.2024.9250295). [3]Brodkorb et al. (2019). *Nature Protocols*, *14*, 991-1014. [4]Makran et al. (2023). *Food & Function*, *14*, 10829-10840.

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