CFAES DEPARTMENT OF ANIMAL SCIENCES

Polyunsaturated Fatty Acids Help Regulate the Inflammatory Response in IPEC J-2 cells Challenged with Enterotoxigenic *E. Coli*

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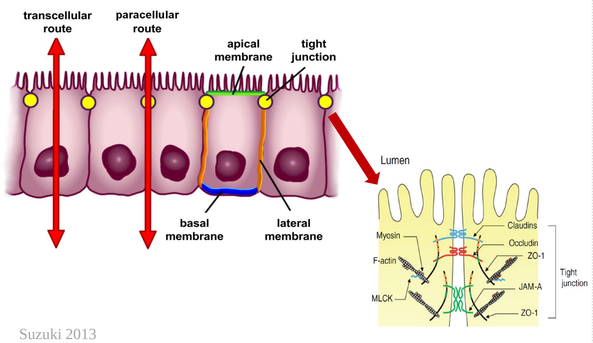
INTRODUCTION

- Intestinal disease is a leading cause of death in neonates.
  - Scours causes ~10% of pre-weaning death loss in the US swine industry (USDA, 2012), and a common cause of the diarrhea is Enterotoxigenic *Escherichia coli* (ETEC) (Nagy, 2005).
  - Additionally, *E. coli* causes 11.6% morbidity in pigs post-weaning (USDA, 2012).
  - ETEC constituents can activate intestinal inflammatory responses and alter gut permeability and intestinal barrier function.
- A greater focus on dietary strategies to optimize a healthy gut environment in animal production will help limit the reliance on antibiotic use.
- Fatty acids (FA), especially polyunsaturated fatty acids (PUFA), have been shown to limit the induction of inflammatory pathways and reduce damage associated with inflammation.
  - Metabolites of PUFA are ligands of the transcription factor PPARγ which helps resolve inflammation through multiple mechanisms.
- The integrity of the intestinal epithelial barrier is crucial to optimizing intestinal function and swine health.
  - Tight junction proteins like claudin-1 are a critical component of the barrier's ability to selectively transport materials across the epithelium.

Intestinal Epithelial Barrier Function



Suzuki 2013

AIM

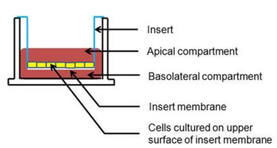
The aim of this research was to identify if increased inclusion of PUFA could enrich the intestinal phospholipid membrane and alter intestinal barrier function. Specifically, how the PUFA arachidonic acid (ARA), eicosapentanoic acid (EPA), docosahexaenoic acid (DHA), and oleate affected transepithelial electrical resistance (TEER) across the intestinal monolayer and claudin-1 protein abundance in neonatal intestinal porcine epithelial cells (IPEC-J2) challenged with ETEC.

HYPOTHESIS

The hypothesis is that dietary PUFA will enrich the phospholipid membrane and, upon challenge, will protect intestinal epithelial barrier function.

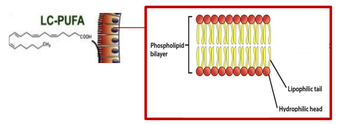
METHODS

- IPEC-J2 cells were plated at a cell density of 5 x 10^4 cells per trans-well and grown to 100% confluence.
  - Cells were supplemented with 30 µM ARA, EPA, DHA or oleate for 96 h.
  - Cells were challenged with 2:1 multiplicity of infection (MOI) of ETEC for 6 h on the apical side.
- Fatty acids were extracted from cells and analyzed using GC/MS.
- TEER readings were recorded during challenge.



- Following the 6 h ETEC challenge, cells were washed with PBS then cell proteins were extracted for analysis of claudin-1 and GAPDH abundance by SDS-PAGE and subsequent Western Blot.
- iBright Analysis Software was used to measure densitometry of Western Blot protein bands.
- Data were analyzed using SAS by ANOVA and means ± SE were reported.
  - Different letters indicate significance $P < 0.05$.

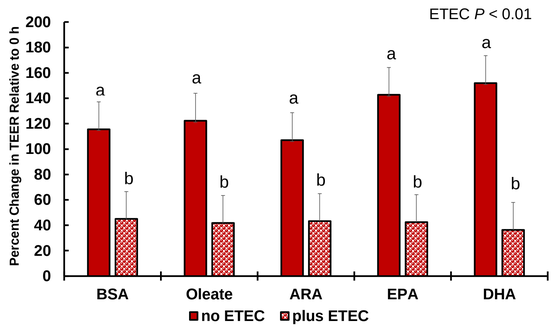
RESULTS



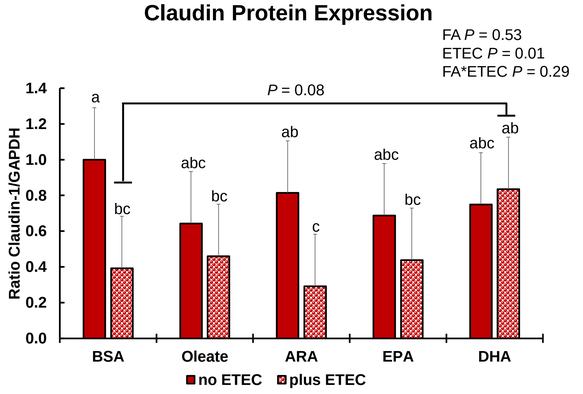
FA Enrichment of IPEC-J2 Phospholipid Membrane

		30 µM Treatment for 96 hours						
		Control	OA	ARA	EPA	DHA	SEM	*P*-value
% Fatty Acid in the membrane	OA	31.65^{ab}	32.26^{a}	29.56^{c}	30.11^{bc}	32.48^{a}	0.89	$P < 0.05$
	ARA	0.96^{b}	0.70^{b}	5.47^{a}	1.02^{b}	0.98^{b}	0.39	$P < 0.05$
	EPA	0.03^{b}	0.18^{b}	0.16^{b}	1.23^{a}	0.23^{b}	0.13	$P < 0.05$
	DHA	0.02^{d}	0.23^{c}	0.26^{c}	0.87^{a}	0.42^{b}	0.11	$P < 0.05$

TEER Six Hours Post ETEC Challenge



Claudin Protein Expression



CONCLUSIONS

- Supplementary PUFA can enrich intestinal epithelial cell phospholipid membranes compared to control cells by:
  - 5.7-fold with ARA
  - 41-fold with EPA
  - 21-fold with DHA
- ETEC at 2:1 MOI significantly alters TEER of the IPEC-J2 intestinal membrane which is consistent with altered ion flux and development of diarrheal disease.
- ETEC challenge reduced tight junction protein abundance in IPEC-J2 cells.
- DHA has a potential protective effect against claudin-1 degradation in IPEC-J2 cells challenged with ETEC compared to BSA challenged cells.

FUTURE RESEARCH

Future research will further investigate the efficacy of dietary supplementation as a preventative method to reduce the damage associated with ETEC in both swine and human health. Moving forward, it would be beneficial to study how fatty acid supplementation affects protein expression of occludin and zona-occluden tight junction proteins, as well as transcription factors of the inflammatory pathway, NF-κB and PPARγ, known to responds to ligand metabolites of PUFA.

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