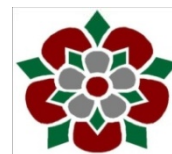


EMU OIL INCREASES CRYPT DEPTH BUT ONLY MINIMALLY AFFECTS OTHER INDICATORS OF COLONIC INTEGRITY IN A RAT MODEL OF COLITIS

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INFLAMMATORY BOWEL DISEASE

- Chronic, idiopathic disease
- Uncontrolled immune response
 - Excess production of pro-inflammatory cytokines (IFN- γ , TNF- α , IL-2)
- *Ulcerative Colitis (UC)* & Crohn's disease
- Current treatments include immunosuppressants, antibiotics, corticosteroids and 5'aminosalicyclic acid → *variably effective*
- Clear need for improved therapeutic approaches

(Hendrickson *et al.*, 2002)



EMU OIL

- Emu (*Dromais Novae-Hollandiae*) = large, flightless bird indigenous to Australia
- Oil extracted from subcutaneous & retroperitoneal fat (*render, filter, centrifuge*) (Whitehouse *et al.*, 1998)
- High fatty acid composition



EMU OIL

FATTY ACID	COMMON NAMES	MEAN (%) (± 1 SD)	RANGE (± 3 SD)
14:0	Myristic	0.4 ± 0.08	0.17 - 0.68
16:0	Palmitic	22.0 ± 1.50	17.5 - 26.5
16:1	Palmitoleic	3.5 ± 0.78	1.2 - 5.7
18:0	Stearic	9.6 ± 0.80	7.2 - 12.0
18:1	Oleic	47.4 ± 3.00	38.4 - 56.4
18:2	Linoleic	15.2 ± 3.00	6.2 - 24.2
18:3	Linolenic	0.9 ± 0.30	0.1 - 1.8



Main anti-inflammatory mediators:

→ Omega 9

→ Omega 3

TGA- compositional guideline: *Refined Emu Oil*

- Also contains variable levels of compounds including antioxidants and skin-permeation enhancing factors

EMU OIL

Evidence of Emu Oil anti-inflammatory properties:

- Yoganathan *et al.*, 2003

- Croton oil-induced auricular swelling in CD-1 mice

- Only Emu Oil significantly reduced auricular thickness and earplug weights (-72% and -71%, respectively)

TABLE 2
Thickness and Weight Differences^a of Ears in Mice Treated
with Various Oils 2 h After Croton Oil Application and 6 h After
Oil Treatment

Treatment	Thickness (mm)	Weight (mg)
Control	0.285 ± 0.023 ^a	24.44 ± 6.45 ^a
Emu oil	0.081 ± 0.009 ^b	7.22 ± 1.45 ^b
Fish oil	0.143 ± 0.013 ^{a,b}	11.22 ± 1.84 ^{a,b}
Flax oil	0.143 ± 0.025 ^{a,b}	13.27 ± 4.13 ^{a,b}
Olive oil	0.171 ± 0.026 ^{a,b}	13.78 ± 1.76 ^{a,b}
Liquified chicken fat	0.205 ± 0.022 ^{a,b}	18.08 ± 2.00 ^{a,b}

^aValues are mean ± SD, *n* = 10. Values in a column not sharing a common superscript roman letter are significantly different at *P* < 0.05.

EMU OIL

Evidence of Emu Oil anti-inflammatory properties:

- Yoganathan *et al.*, 2003
 - Emu Oil significantly reduced pro-inflammatory mediators (TNF- α , IL-1 α)
 - Greater reduction by Emu Oil *cf.* fish, flaxseed, olive, or liquefied chicken fat, or left untreated

TABLE 3

Ear Plug Tissue Concentrations^a of IL-1 α and TNF- α in Mice Treated with Various Oils 2 h After Croton Oil Application and 6 h After Oil Treatment

Treatment	IL-1 α (pg/mg)	TNF- α (pg/mg)
Control	307.2 \pm 35.02 ^a	79.25 \pm 15.53 ^a
Emu oil	92.3 \pm 12.18 ^b	31.74 \pm 3.62 ^b
Fish oil	132.2 \pm 19.65 ^b	50.67 \pm 10.17 ^{a,b}
Flax oil	173.9 \pm 40.95 ^{a,b}	52.61 \pm 7.14 ^{a,b}
Olive oil	155.9 \pm 27.38 ^{a,b}	38.27 \pm 5.23 ^b
Liquified chicken fat	227.7 \pm 23.13 ^{a,b}	56.85 \pm 6.19 ^{a,b}

^aValues are mean \pm SD, $n = 10$. Values in a column not sharing a common superscript roman letter are significantly different at $P < 0.05$. IL, interleukin; TNF- α , tumor necrosis factor-alpha.

EMU OIL

- Yoganathan *et al.*, 2003

- Emu Oil significantly reduced pro-inflammatory mediators (TNF- α , IL-1 α)
- Greater reduction by Emu Oil *cf.* fish, flaxseed, olive, or liquefied chicken fat, or left untreated
 - unusual: Emu Oil contains significantly less anti-inflammatory FAs than other oils
 - thus, Emu Oil anti-inflammatory properties are not fully explained by the FA profile



HYPOTHESIS

Emu Oil would decrease the severity of dextran sulphate sodium (DSS)-induced colitis in the rat through a preventative and/or regenerative mechanism.



AIMS

1. To evaluate Emu Oil for its potential to ameliorate DSS-induced colitis when administered orally to rats
2. To compare its potential protective or reparative properties



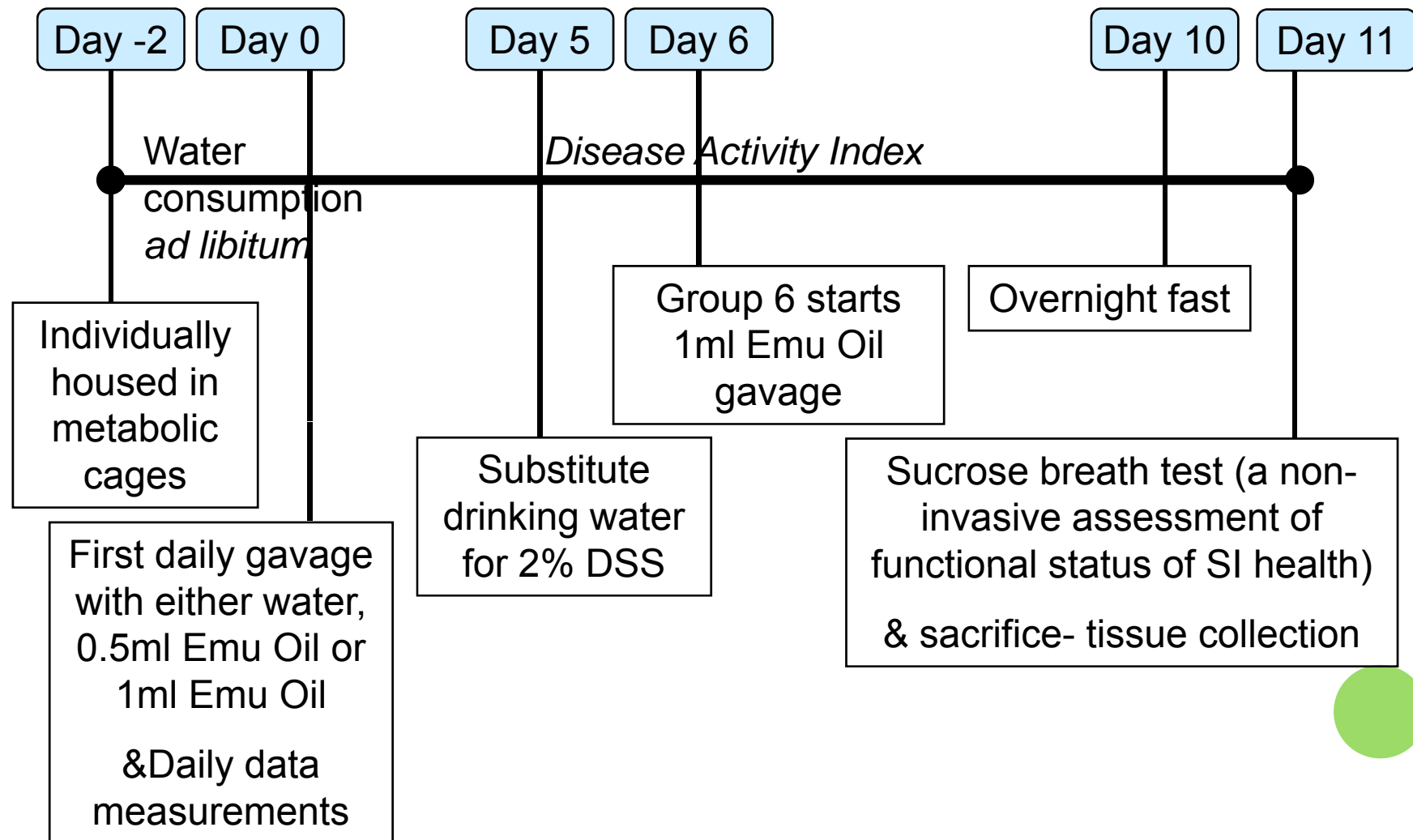
RESEARCH PLAN

- Male Sprague Dawley rats (135-150g)
- 11 day trial
- 6 treatment groups (n=8/group)

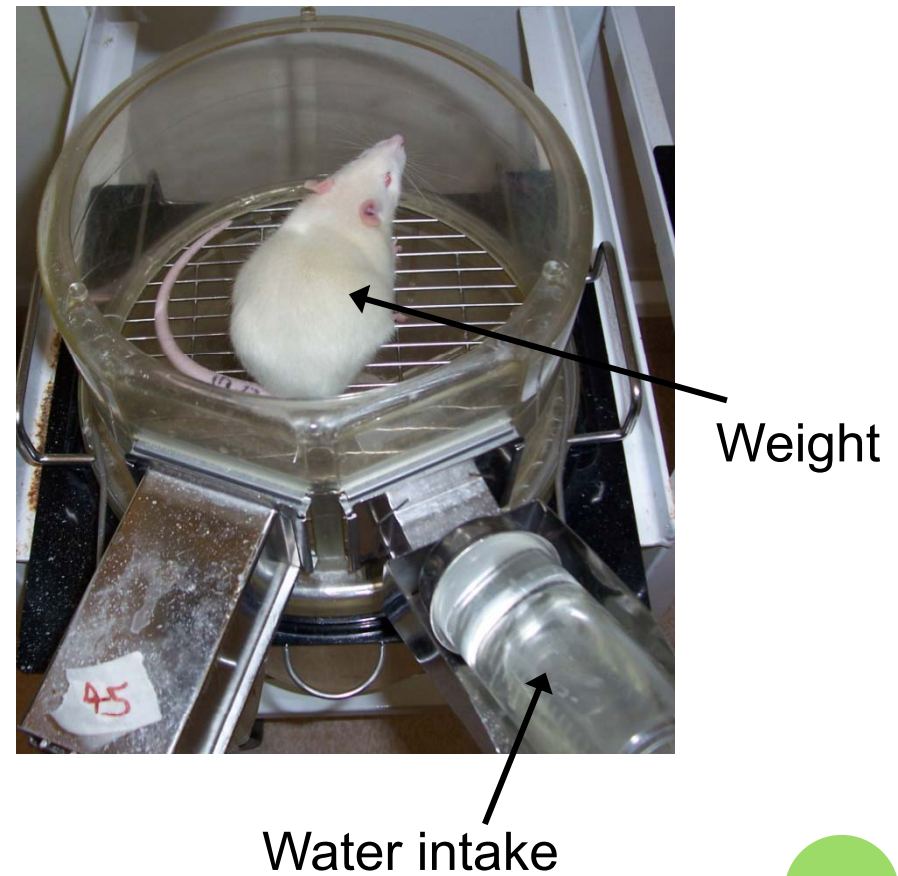
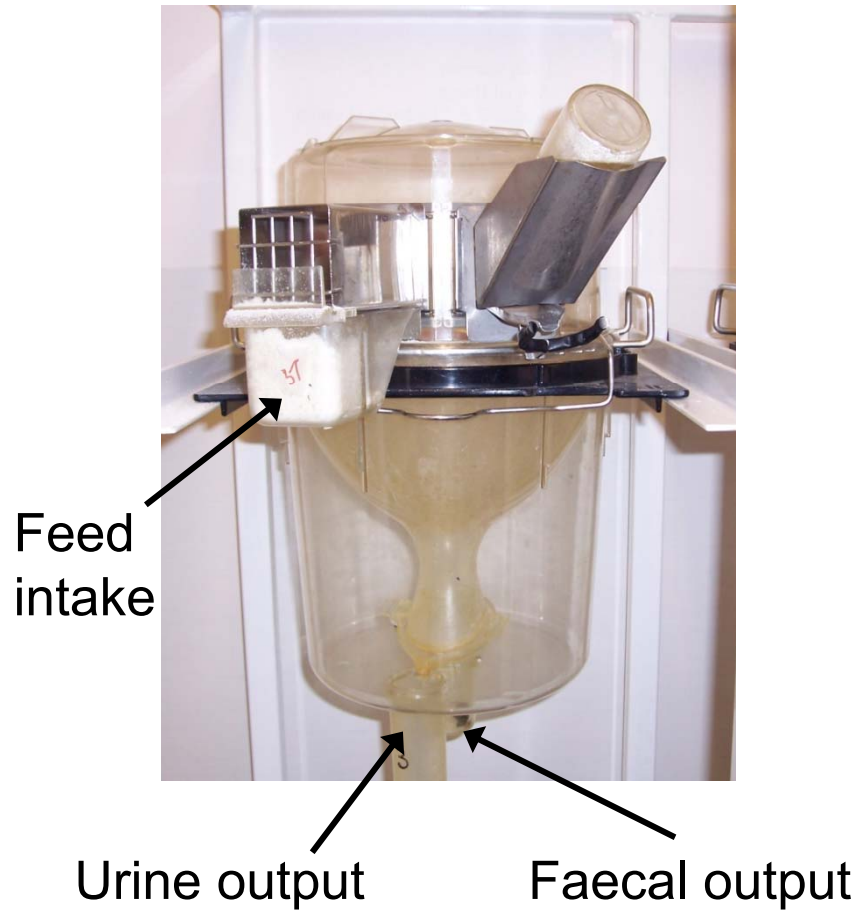
ad libitum : oral gavage

- 1: Water + Water
2. Water + 1ml Emu Oil
- 3: DSS + Water
- 4: DSS + 0.5ml Emu Oil
- 5: DSS + 1ml Emu Oil
- 6: DSS + 1ml Emu Oil at day 6 (1 day post DSS commencement)

RESEARCH PLAN



DAILY MEASUREMENTS



HISTOLOGICAL ANALYSES

- 4µm sections of colon were stained with Haematoxylin & Eosin to measure:
 - Proximal and Distal Colonic crypt depth
 - Proximal and Distal Colonic Overall Damage severity score (8 parameters)



Representative photomicrographs of 4µm sections of distal colon stained with H&E.
(a) Healthy **(b)** Colitic controls

BIOCHEMICAL ANALYSIS

- Myeloperoxidase (MPO) assay
- MPO= enzyme in intracellular granules of neutrophils
 - Tissue neutrophil content index
 - Indirect measure of acute inflammation in colon
(Mauger *et al.*, 2007)



RESULTS



RESULTS

Emu Oil had no significant effect on:

- Disease Activity Index
- Daily data (feed and water intake, faecal and urine output)
- Sucrose Breath Test
- Body weight change
- Myeloperoxidase activity (acute inflammation)



RESULTS

Emu Oil significantly:

- Decreased damage severity *cf.* Colitic-controls
- Lengthened proximal and distal colonic crypts



CONCLUSIONS

1. Emu Oil improved selected biological parameters associated with damage to the intestine in an experimental model of colitis
2. This may represent a new mechanism of action for Emu Oil in protection and repair from injury, indicating its therapeutic potential as a dietary supplement to augment conventional treatment approaches for IBD



FUTURE DIRECTIONS

- Other bowel conditions (mucositis; NSAID-enteropathy)
- Comparisons with other ratite oils and animal oils
- Inter-batch variations
- Optimal dose and timing for treatment regimens
- Fractionation- identification of the active factor



ACKNOWLEDGEMENTS

THE WOMEN'S AND CHILDREN'S HOSPITAL, NORTH ADELAIDE, SA

Assoc. Prof. Gordon Howarth

Assoc. Prof. Ross Butler

Ruth Lindsay

Kerry Lymn

Gastro lab

THE QUEEN ELIZABETH HOSPITAL, WOODVILLE NORTH, SA

Dr. Adrian Cummins

Basil Hetzel Institute

Gastro lab

EMU TRACKS PTY LTD, MARLESTON, SA, AUSTRALIA

THE UNIVERSITY OF ADELAIDE

WORLD CONGRESS ON OILS AND FATS & 28th ISF CONGRESS 2009

