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

SUZANNE MASHTOUB

Discipline of Physiology, School of Molecular & Biomedical Science, The University of Adelaide, SA, Australia

suzanne.mashtoub@adelaide.edu.au
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 \( \rightarrow \text{variably effective} \)
- 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

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

TGA- compositional guideline: *Refined Emu Oil*

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

Main anti-inflammatory mediators:

- **Omega 9**
- **Omega 3**
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>
<thead>
<tr>
<th>Treatment</th>
<th>Thickness (mm)</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.285 ± 0.023(^a)</td>
<td>24.44 ± 6.45(^a)</td>
</tr>
<tr>
<td>Emu oil</td>
<td>0.081 ± 0.009(^b)</td>
<td>7.22 ± 1.45(^b)</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0.143 ± 0.013(^a,b)</td>
<td>11.22 ± 1.84(^a,b)</td>
</tr>
<tr>
<td>Flax oil</td>
<td>0.143 ± 0.025(^a,b)</td>
<td>13.27 ± 4.13(^a,b)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.171 ± 0.026(^a,b)</td>
<td>13.78 ± 1.76(^a,b)</td>
</tr>
<tr>
<td>Liquified chicken fat</td>
<td>0.205 ± 0.022(^a,b)</td>
<td>18.08 ± 2.00(^a,b)</td>
</tr>
</tbody>
</table>

\(^a^Values 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-1α)
  - Greater reduction by Emu Oil *cf.* fish, flaxseed, olive, or liquefied chicken fat, or left untreated

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-1α (pg/mg)</th>
<th>TNF-α (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>307.2 ± 35.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.25 ± 15.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Emu oil</td>
<td>92.3 ± 12.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.74 ± 3.62&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish oil</td>
<td>132.2 ± 19.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.67 ± 10.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flax oil</td>
<td>173.9 ± 40.95&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>52.61 ± 7.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Olive oil</td>
<td>155.9 ± 27.38&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>38.27 ± 5.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liquified chicken fat</td>
<td>227.7 ± 23.13&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>56.85 ± 6.19&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean ± 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-1α)
- 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**

Day -2
- Water consumption *ad libitum*

Day 0
- Individually housed in metabolic cages

Day 5
- First daily gavage with either water, 0.5ml Emu Oil or 1ml Emu Oil
- Substitute drinking water for 2% DSS

Day 6
- Group 6 starts 1ml Emu Oil gavage
- Disease Activity Index

Day 10
- Overnight fast

Day 11
- Sucrose breath test (a non-invasive assessment of functional status of SI health)
- & sacrifice- tissue collection

&Daily data measurements
DAILY MEASUREMENTS

Feed intake

Urine output

Faecal output

Water intake

Weight
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-eneteropathy)
- 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
Ruth Lindsay
Gastro lab

Assoc. Prof. Ross Butler
Kerry Lymn

THE QUEEN ELIZABETH HOSPITAL, WOODVILLE NORTH, SA

Dr. Adrian Cummins
Gastro lab

Basil Hetzel Institute

EMU TRACKS PTY LTD, MARLESTON, SA, AUSTRALIA

THE UNIVERSITY OF ADELAIDE

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