Suplements de plasma animal en l’alleugeriment de malalties inflamatòries

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Weaning and the consequences of stress

Animal blood plasma → Spray-drying → SDP

SDP in a model of mild intestinal inflammation
SDP in a model of acute lung inflammation
SDP in a model of colitis

The mechanism(s) of action of SDP
Intestinal mucosa

Background

Gut associated lymphoid tissue

Background

Weaning

- Transient anorexia
- Enteric infections & diarrhoea
- Intestinal malabsorption
- Delay in the anatomical and functional development of the mucosal immune system
- Decrease in barrier function
- Disturbancies in the homeostasis of gut microbiota

Villus atrophy
**SDP supplementation**

**Animal blood plasma**

**Typical SDP composition**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>SDPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>69.1</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>13.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.9</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>-</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>-</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>10.6</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>4.43</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>17</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>101</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>5</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>9</td>
</tr>
</tbody>
</table>


**Piglets post-weaning**

Stimulates growth performance and feed intake

Remus et al 2013, Livestock Sci 155: 294-300

Reduces post-weaning diarrhoea
**SEB model: EXPERIMENTAL DESIGN**

Inflammatory agent: Entorotoxin B from *Staphylococcus aureus* (SEB)

- C57/BL6 mice
- Wistar-Lewis rats

**SEB challenge**
0.5 mg/kg (i.p.)

**On diet (14 days)**

**SEB-SDP:** SDP* suppl. (8%) + SEB

**Sacrifice**
- Mice (Day 33)
- Rats (Day 35)

*Supplied by APC

**Intestinal inflammation**

**SEB**

**APC**

**Polyclonal T-lymphocyte activation**
SEB model: SUMMARY

- **Luminal water contents**
- **Epithelial permeability**
  - Flux of dextran and HRP and tight junction proteins
- **Mucosal defensins**
  - Cryptdin 4 and β-defensin 1 expression
- **Organized GALT (PP, MLN)**
  - Recruitment of cytotoxic populations
- **Diffuse GALT (LPL, IEL)**
  - Recruitment of cytotoxic populations
- **Mucosal cytokines**
  - Pro-inflammatory cytokine release
Spray-Dried Animal Plasma Prevents the Effects of *Staphylococcus aureus* Enterotoxin B on Intestinal Barrier Function in Weaned Rats

Anna Pérez-Bosque, Concepción Amat, Javier Polo, Joy M. Campbell, Joe Crenshaw, Louis Russell, and Miquel Moretò

**Intestinal permeability**

**Spray-Dried Animal Plasma Prevents the Effects of *Staphylococcus aureus* Enterotoxin B on Intestinal Barrier Function in Weaned Rats**

SEB model: RESULTS

Cytokines

Intestinal mucosa

TNF-α

IL-10

Control
SEB
SEB-SDP

Effects of SEB
SEB + SDP supplementation

Plaques de Peyer

Serum

TNF-α

IL-10

Control
SEB
SEB-SDP

Luminal water contents

Epithelial permeability
  • Flux of dextran and HRP and tight junction proteins

Mucosal defensins
  • Cryptdin 4 and β-defensin 1 expression

Organized GALT (PP, MLN)
  • Recruitment of cytotoxic populations

Diffuse GALT (LPL, IEL)
  • Recruitment of cytotoxic populations

Mucosal cytokines
  • Pro-inflammatory cytokine release

Plasma proteins can modulate the degree of GALT activation, restoring the barrier functions of the intestinal mucosa
Oral SDP supplements:
- Increase performance of poult's the first week after placement.
- Reduced mortality in turkeys exposed to *Pasteurella multocida*.

LPS model: EXPERIMENTAL DESIGN

Inflammatory agent: Lipopolysaccharide from *E. coli*

C57BL/6 mice

Weaning (day 19)

ON DIET (14 days)

LPS challenge
12.5 µg i.n. (day 33)

Sacrifice
(6 h / 24 h after the challenge)

**CTL:** Control diet

**LPS:** Control diet + LPS

**LPS-SDP:** SDP* suppl. (8%) + LPS

*Supplied by [APC](#)

- Brochoalveolar lavage fluid (BALF)
- Lung tissue
- Blood
Colitis model: SUMMARY

**Innate immunity**
- Activated monocytes and neutrophiles, pro-inflammatory cytokines & chemokines.

**Adaptive immunity**
- Activated Th lymphocytes, pro-inflammatory cytokines.

**Regulatory immunity**
- Treg lymphocytes, anti-inflammatory cytokines.
LPS model: RESULTS

Cytokines

**TNF-α concentration**

- **Non challenged:**
  - 6 h: Baseline
  - 24 h: Increased

- **Challenged:**
  - 6 h: Increased
  - 24 h: Decreased

**IFN-γ concentration**

- **Non challenged:**
  - 6 h: Baseline
  - 24 h: Increased

- **Challenged:**
  - 6 h: Increased
  - 24 h: Decreased

**IL-10 concentration**

- **Control:**
  - 6 h: Baseline
  - 24 h: Increased

- **LPS:**
  - 6 h: Decreased
  - 24 h: Increased

- **LPS-SDP:**
  - 6 h: Increased
  - 24 h: Baseline

**Maijó et al. (2012) Br J Nutr 107:867-75**

**Maijó et al. (2012) J Nutr 142:264-70**
Colitis model: SUMMARY

+ SDP

**LPS**

**Innate immunity**
- Activated monocytes and neutrophiles, pro-inflammatory cytokines & chemokines.

**Adaptive immunity**
- Activated Th lymphocytes, pro-inflammatory cytokines.

**Regulatory immunity**
- Treg lymphocytes, anti-inflammatory cytokines.

Dietary plasma proteins reduce the immune response that characterizes the acute lung inflammation syndrome
Outline

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The mechanism(s) of action of SDP
Background

Colon mucosa

Colitis model: EXPERIMENTAL DESIGN

**SPF area (4 wk)**
- **Birth**
- **Weaning**
- **Sacrifice**

**Conventional area (4 wk)**

Week 1 2 3 4 5 6 7 8

**The model**

- **P-glycoprotein**
- **Drug efflux**
- **ATP-binding domain**
- **Plasma membrane**
- **Xenobiotics**

**On diet (5 wk)**
- **WT-CTL**: Control diet
- **KO-CTL**: Control diet
- **KO-SDP**: SDP suppl. diet (8%)*
  
  *Supplied by

- P-glycoprotein is expressed in the apical membrane of enterocytes.
- It pumps xenobiotics back into the intestinal lumen.
- In the absence of P-glycoprotein, bacterial products and toxins can cause damage to the intestinal barrier and subsequently initiating the inflammatory process.
Colitis model: EXPERIMENTAL DESIGN

**SPF area (4 wk)**

- Week 1: Birth
- Week 2: Weaning
- Week 3: SPF area (4 wk)
- Week 4: Conventional area (4 wk)
- Week 5: Sacrifice

**On diet (5 wk)**

- **WT-CTL**: Control diet
- **KO-CTL**: Control diet
- **KO-SDP**: SDP suppl. diet (8%)*

*Supplied by

**The model**

- Bloody rectal discharge
- Rectum ulcers
- MLN hyperplasia
- Colon distension
- Shorter and thicker colon
- WT mdra1+/+
- KO mdra1-/-

**mdr1a -/- mouse**
**Colitis model: RESULTS**

**Histopathological index**

### Variables considered
- Lamina propria cell infiltration
- Transmural cell infiltration
- Cryptitis
- Mucosal ulceration
- Crypt abscess formation
- Goblet cell depletion

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**Histopathological index**

**Scores**

- **WT-CTL**: Score
- **KO-CTL**: Score
- **KO-SDP**: Score

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Colitis model: RESULTS

Cell populations

Activated cells in MLN

% of Th lymphocytes

Treg in MLN

% of Th lymphocytes

Tact/Treg in MLN

% of Th lymphocytes

Activated cells in LP

% of Th lymphocytes

Treg LPL

% of Th lymphocytes

Tact/Treg in LP

% of Th lymphocytes

Moretó et al 2010 Dig Dis Week Meeting, New Orleans, LA.
Colitis model: RESULTS

**Cytokines**

**TNF-α expression**

- WT
- KO
- KO-SDP

**IFN-γ expression**

- WT
- KO
- KO-SDP

**IL-2 in colon**

- WT
- KO
- KO-SDP

**IL-10 concentration**

- WT
- KO
- KO-SDP

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Moretó et al 2010 Dig Dis Week Meeting, New Orleans, LA.
Colitis model: RESULTS

Crypt permeability

FITC-Dextran

Crypt lumen

Pericryptal space

FITC Dextran flux

0 min

6 min

12 min

Slope = Permeability index (min⁻¹)

Colitis model: RESULTS

Mucin expression

Histopathological index

Organized GALT (MLN)
  • Recruitment of cytotoxic populations

Diffuse GALT (LPL, IEL)
  • Recruitment of cytotoxic populations

Mucosal cytokines
  • Pro-inflammatory cytokine release

Apical junctional complex
  • Expression of β-catenin and E-cadherin

Epithelial crypt permeability
  • Flux of dextran

Mucosal mucins
  • Expression of MUC2 & TFF3
Colitis model: SUMMARY

Histopathological index

Organized GALT (MLN)
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Plasma proteins can modulate the degree of GALT activation, restoring the barrier functions of the colonic epithelium.
In animal models of inflammatory diseases, dietary supplementation with animal Spray Dried Plasma, by mechanisms involving the luminal-mucosal crosstalk and the participation of GALT and CMIS, can induced changes in physiological variables that can alleviate disease.
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The mechanism(s) of action of SDP
Mecanism of action

Gut lumen

SDP

1. Reduction in luminal antigens
2. Prebiotic effects
3. Bioactive components

IEL
DC

LPL

GALT

Modulates intestinal inflammation

CMIS
Other mucosal regions

Pro- / Anti-inflammatory cytokines
Inflammatory cell recruitment