Immune response to phytonutrients in pigs – antioxidant response

Yanhong Liu

University of California, Davis

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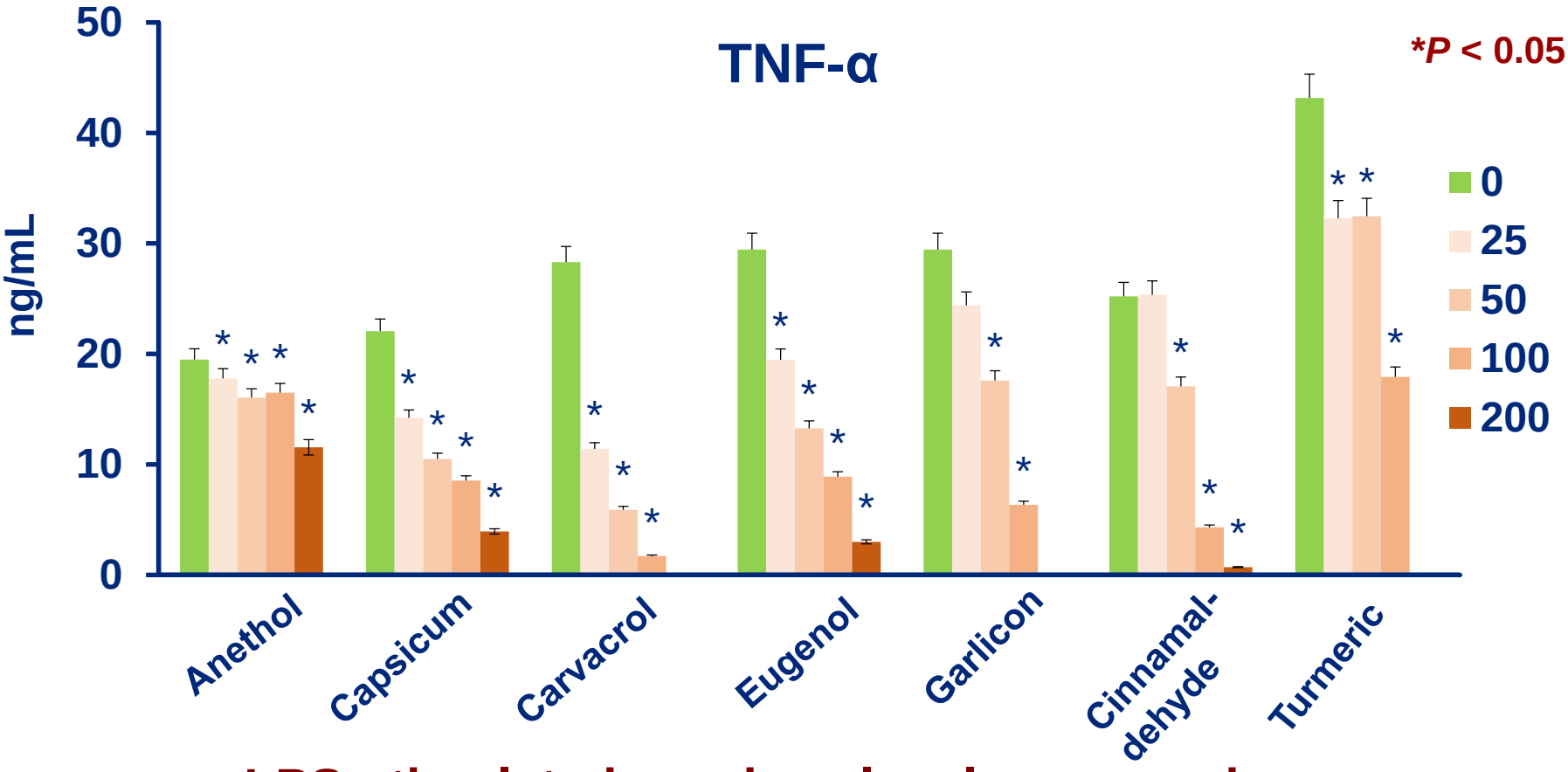
Outline

- Phytonutrients – plant extracts
  - Anti-inflammatory effects
- Weaning stress – oxidative stress
- Antioxidants – plant extracts?
- Overall summary
- Future research

Phytonutrients- plant extracts

- Extracted from parts of plants or synthesized
- Concentrated, hydrophobic, volatile aroma
- Mixtures of secondary plant metabolites
- Liquid or powder
- Phenolic compounds

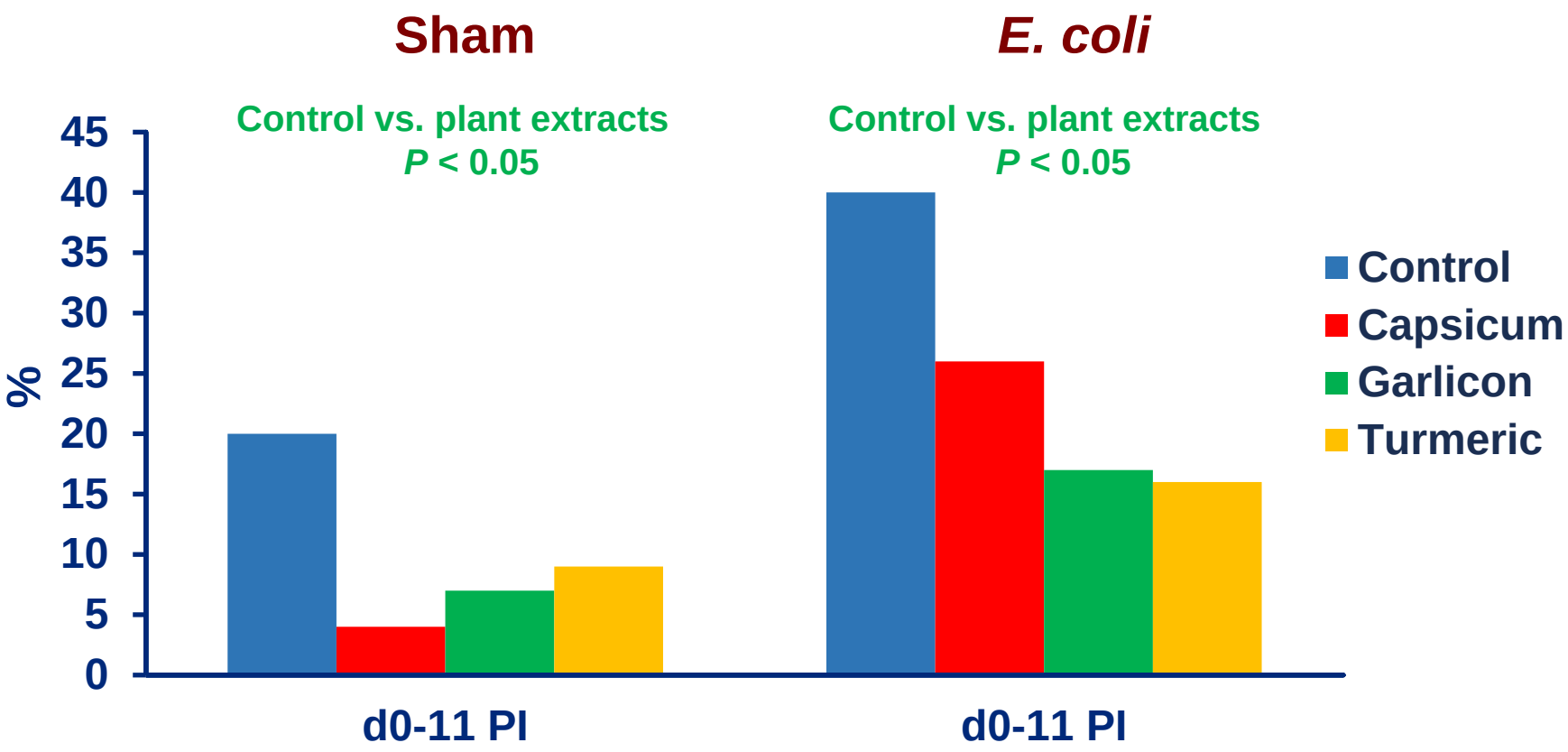
Anti-inflammatory effects
In vitro



LPS-stimulated porcine alveolar macrophages

Liu et al., 2012

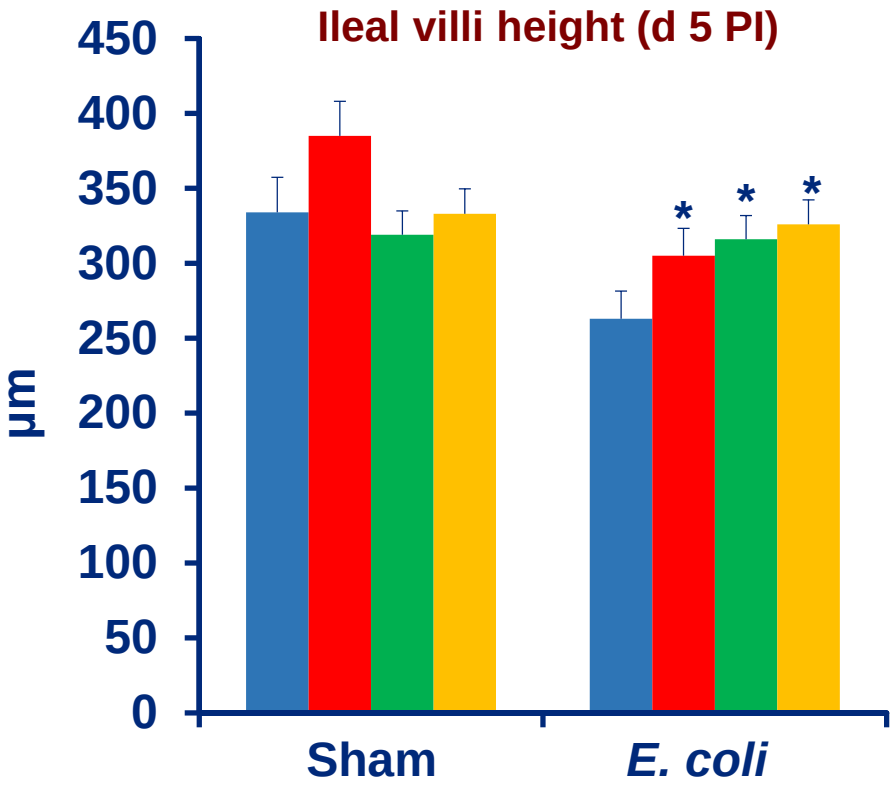
Frequency of diarrhea

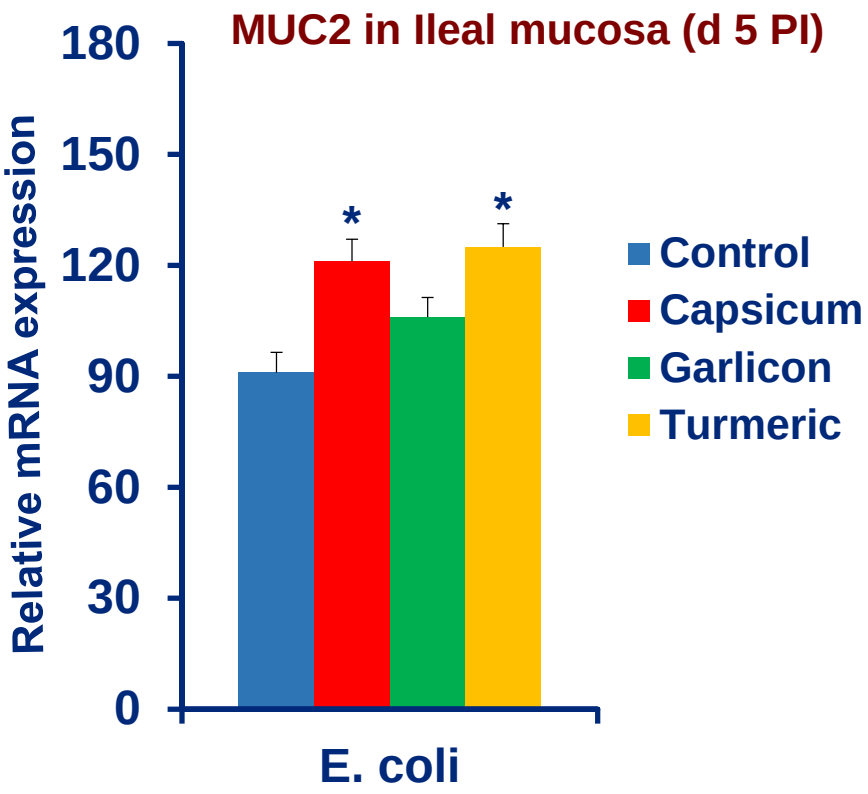


Pig days with diarrhea score ≥ 3
1, normal; 5, watery diarrhea

Liu et al., 2013

Possible mechanism for reduced diarrhea

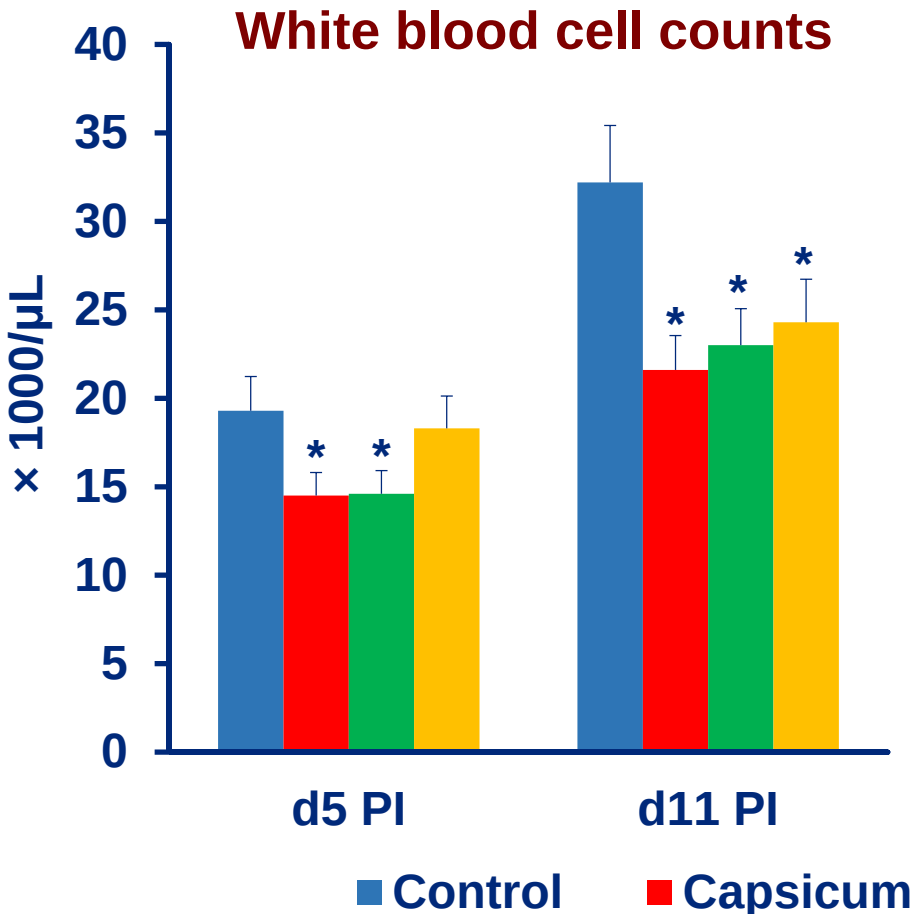


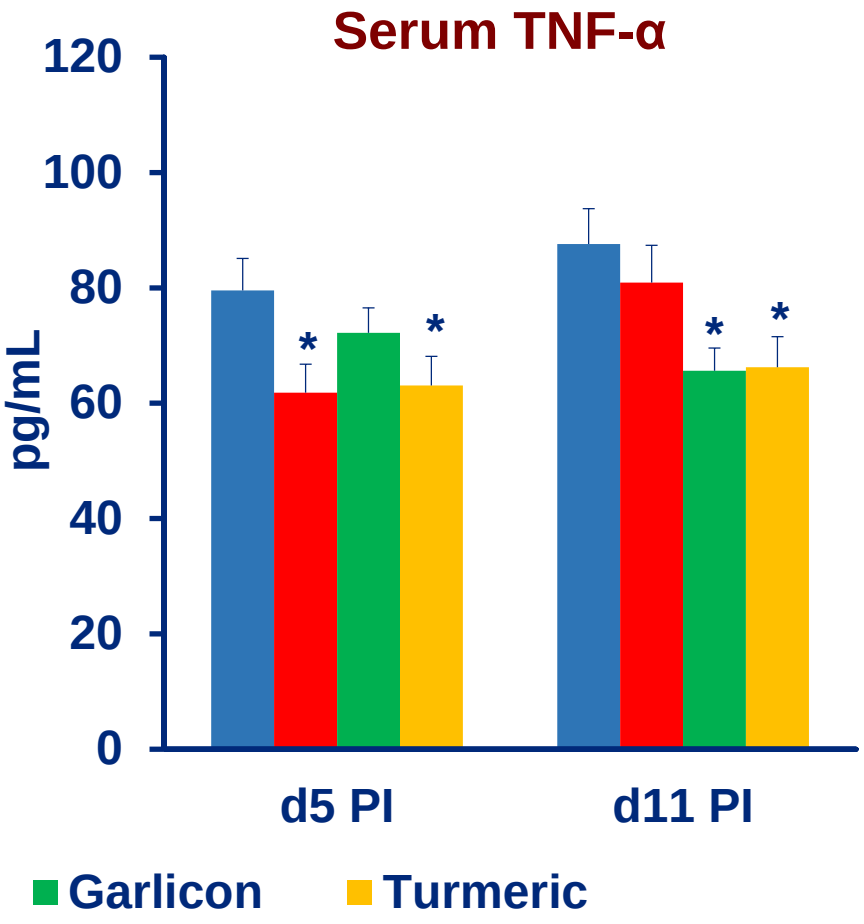


- **Possibly improved gut barrier function!**

Liu et al., 2013, 2014

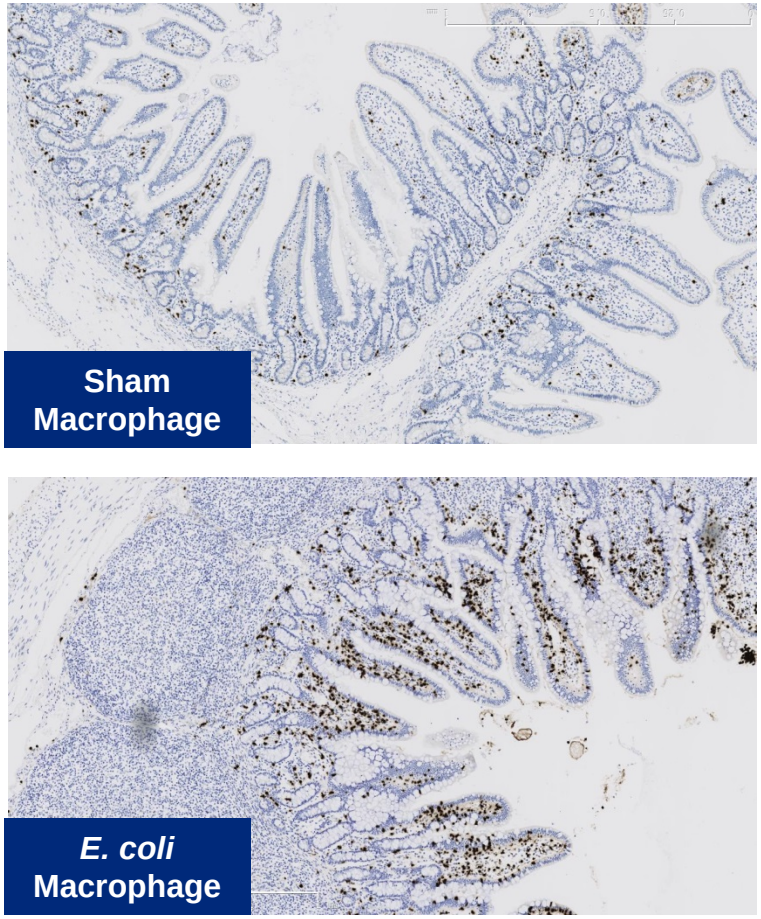
Plant extracts reduced systemic inflammation caused by *E. coli* infection





Liu et al., 2013

Plant extracts reduced gut inflammation caused by *E. coli* infection



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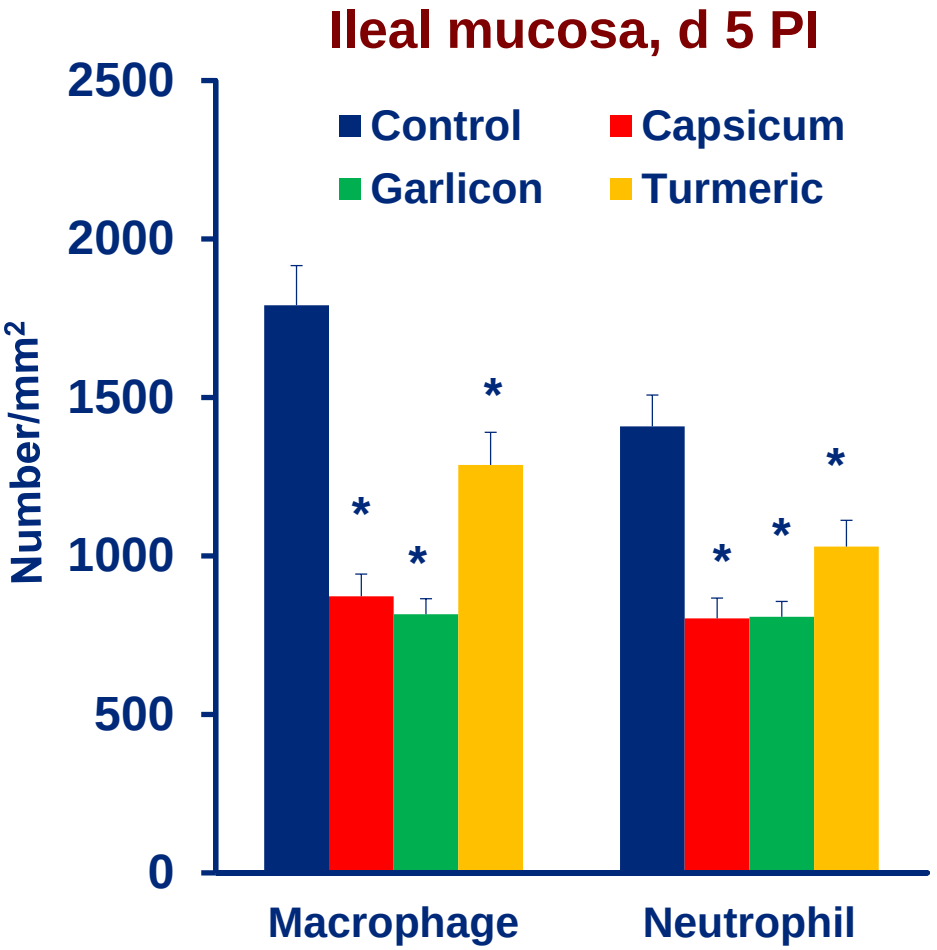
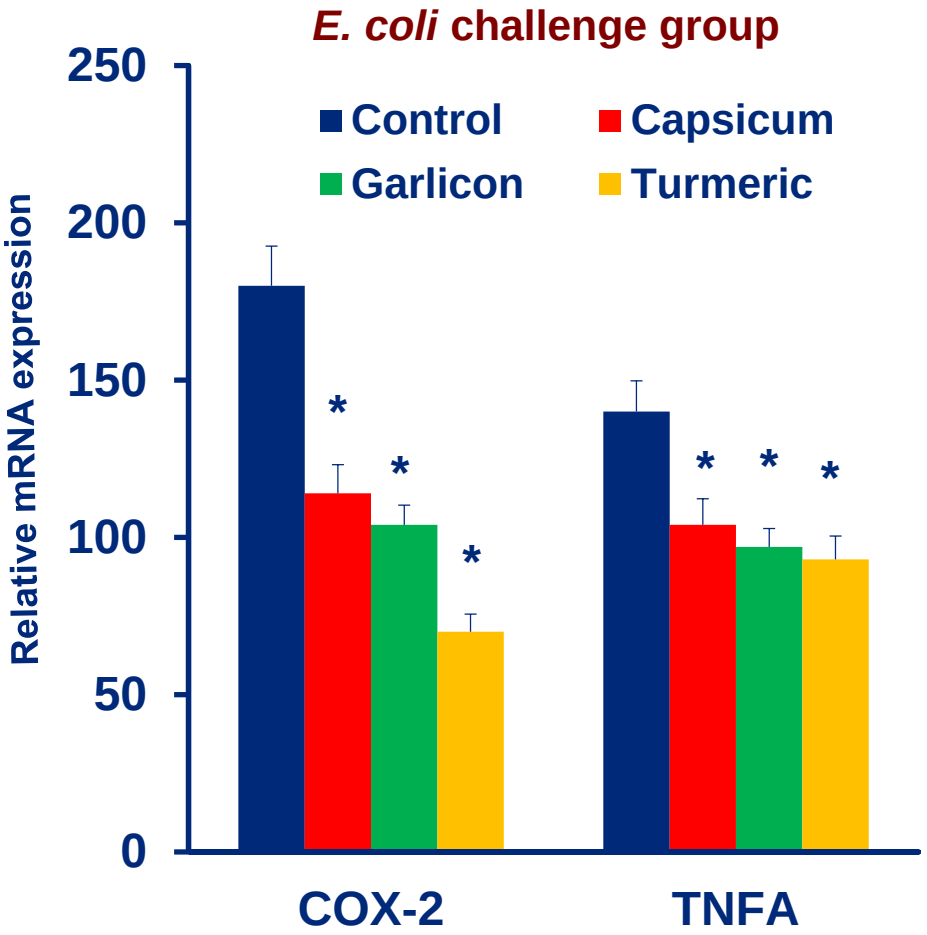
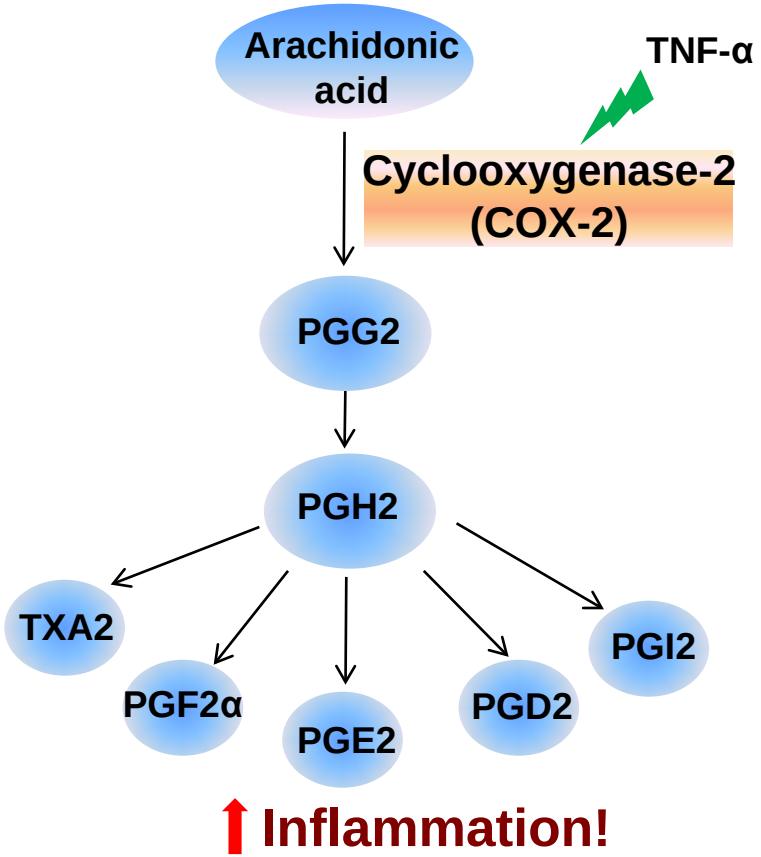


Liu et al., 2013

Plant extracts reduced gut inflammation caused by *E. coli* infection



The Prostaglandin Pathway



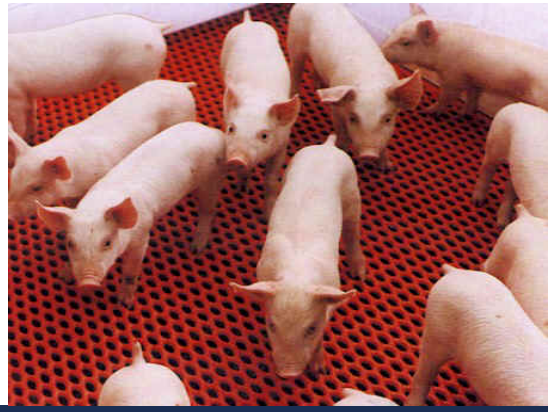
Liu et al., 2014

Summary
Anti-inflammatory effects

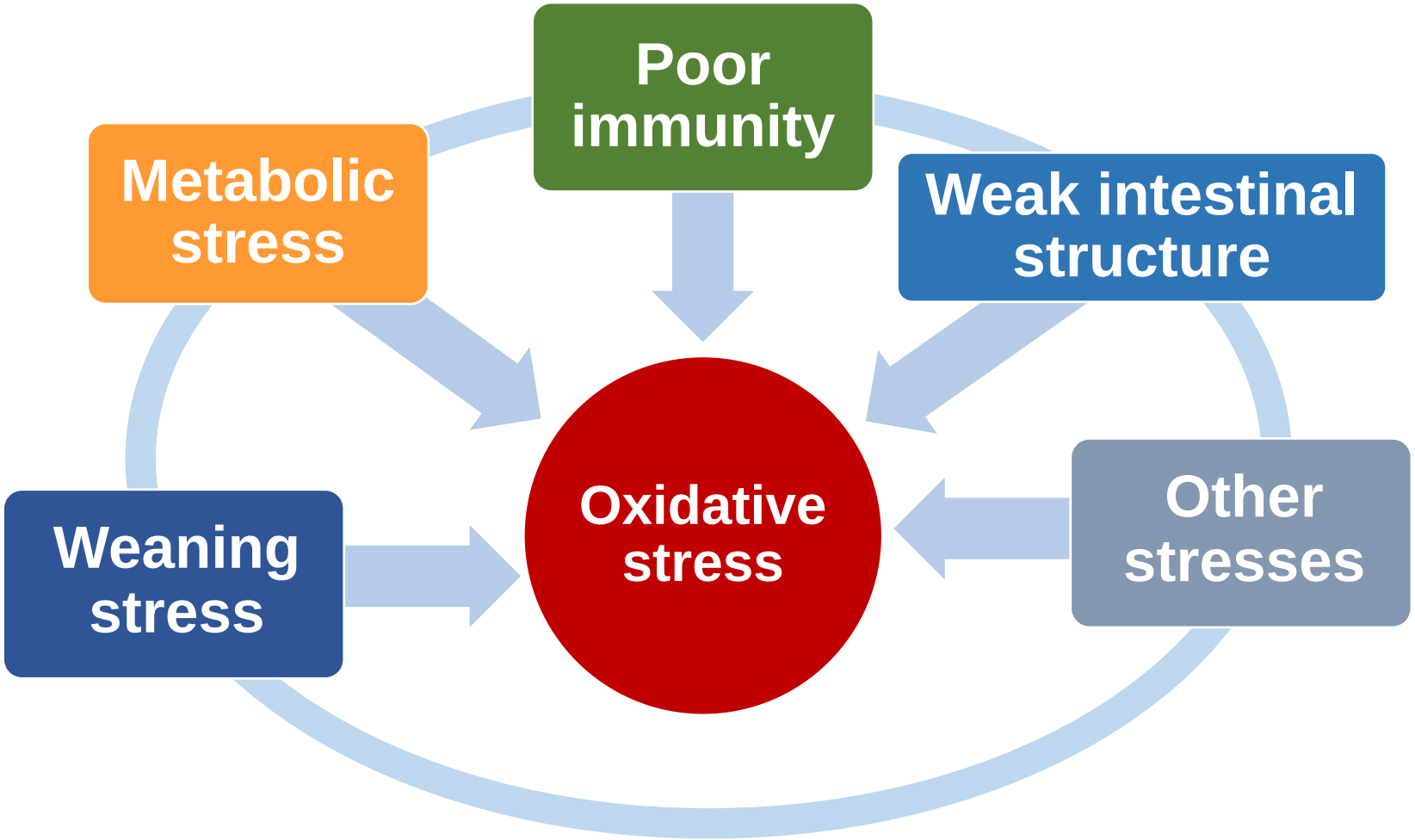
- Suppressed the production of inflammatory mediators in vitro
- Reduced diarrhea and enhanced disease resistance of weaning pigs
- Possible mechanisms
  - Gut barrier function
  - Gut mucosa immunity
  - Systemic immunity
  - Reduced oxidative stress?

Weaning stress

- Maternal separation
- Environmental change
- Increased exposure to pathogens
- Social hierarchy stress
- Move to solid feed
- Transportation stress



Oxidative stress in weaning pigs

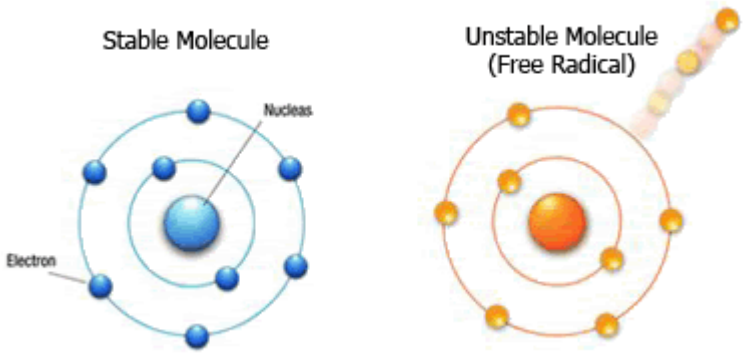


Oxidative stress

- **An excessive production of reactive oxygenated species that cannot be counteracted by the action of antioxidants (Pisoschi and Pop, 2015)**
- **A disturbance in the prooxidant to antioxidant balance in favor of the oxidant species, leading to potential damage (Sies et al., 1991)**

Reactive oxygen species (ROS)

- **Free radical and non-free radical oxygen molecules**
  - **Hydrogen peroxide (H_2O_2)**
  - **Superoxide (O_2-)**
  - **Singlet oxygen (1/2 O_2)**
  - **Hydroxyl radical (·OH)**



McDaniel, 2013

Reactive oxygen species (ROS)

- **Internally generated sources**
  - **Mitochondria**
  - **Xanthine oxidase**
  - **Peroxisomes**
  - **Inflammation**
  - **Phagocytosis**
  - **Arachidonate pathways**
  - **Injury**

Valko et al., 2006

Reactive oxygen species (ROS)

- **Dual roles in biological system**
  - **Low concentration - defend against infectious agents**
  - **High concentration – important mediators of damage to cell structures, including lipids, proteins, and nucleic acids**
  - **Balance is very important !**

Valko et al., 2006

Biomarkers for oxidative stress

Free radicals acceleration	• H_2O_2, NO
Antioxidant status	• Tocopherols, ascorbic acid, uric acid • Glutathione (GSH and GSSG), etc.
Antioxidant enzyme activities	• Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), 8-hydroxyl-2-deoxyguanosine (8-OHdG), catalase (CAT), Inhibitory hydroxyl ability (IHA)
Lipid peroxidation	• Malondialdehyde (MDA)

Kadiiska et al., 2015

Systemic oxidative stress caused by weaning

- Increased free radicals in serum
  - H_2O_2, NO
- Reduced antioxidant enzyme activities in serum
  - GSH-Px, SOD
- Increased lipid peroxidation in serum
  - MDA

Zhu et al., 2013

Oxidative stress in GI tract caused by weaning

- Reduced digestive enzyme activities in jejunum
  - Sucrase, Maltase, Amylase, Lipase
- Increased caspase concentrations in jejunum
  - Caspase-3, caspase-8, caspase-9
- Increased lipid peroxidation and decreased antioxidant enzymes activities

Zhu et al., 2012, 2013

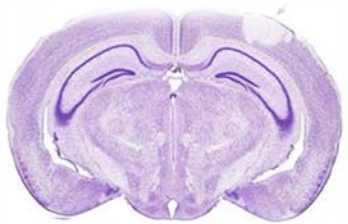
Oxidative stress in liver caused by weaning

- Increased free radicals
  - H_2O_2, NO
- Increased oxidative injury
  - MDA, 8-OHdG
- Reduced antioxidant enzyme activities
  - GSH-Px, SOD, IHA
- Enhanced hepatic enzyme activities
  - Aspartate aminotransferase (AST), alanine aminotransferase (ALT)

Luo et al., 2016

Oxidative stress in brain

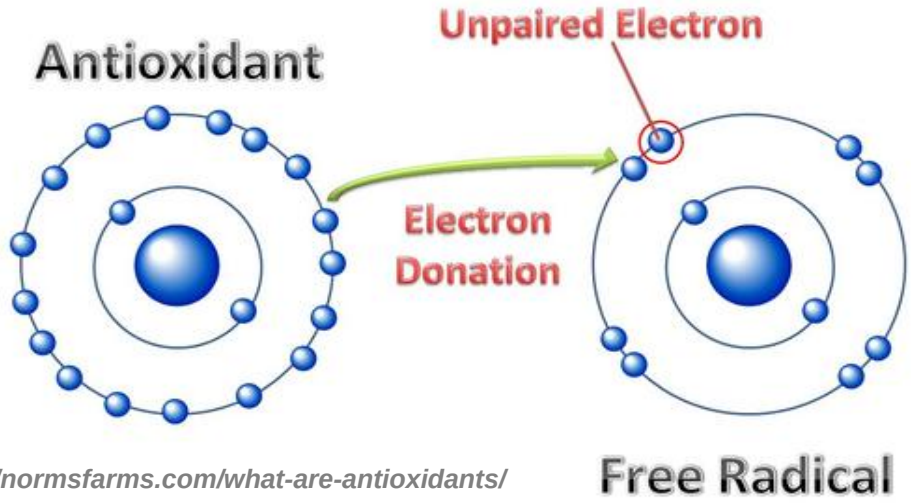
- Increased lipid peroxidation
- Decreased GSH level and GSH/GSSG ratio
- Reduced antioxidant enzyme activities
  - IHA, SOD, GSH-Px, CAT
- Rat data, need verify in pigs



Hong et al., 2016

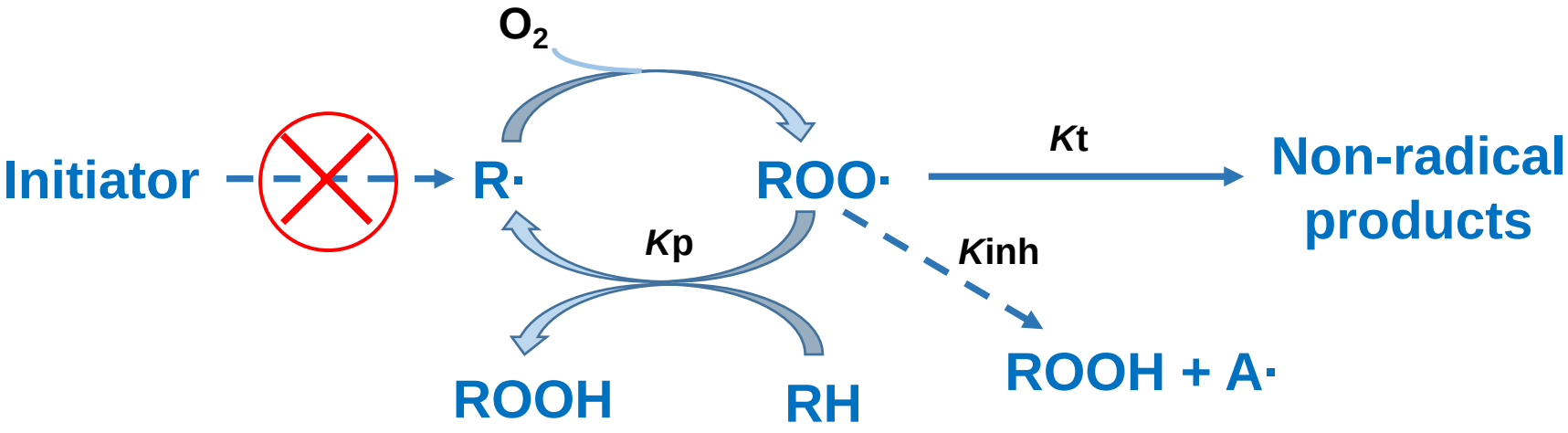
Antioxidants

- **Stable molecules, donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage** **(Lobo et al., 2016)**



http://normsfarms.com/what-are-antioxidants/

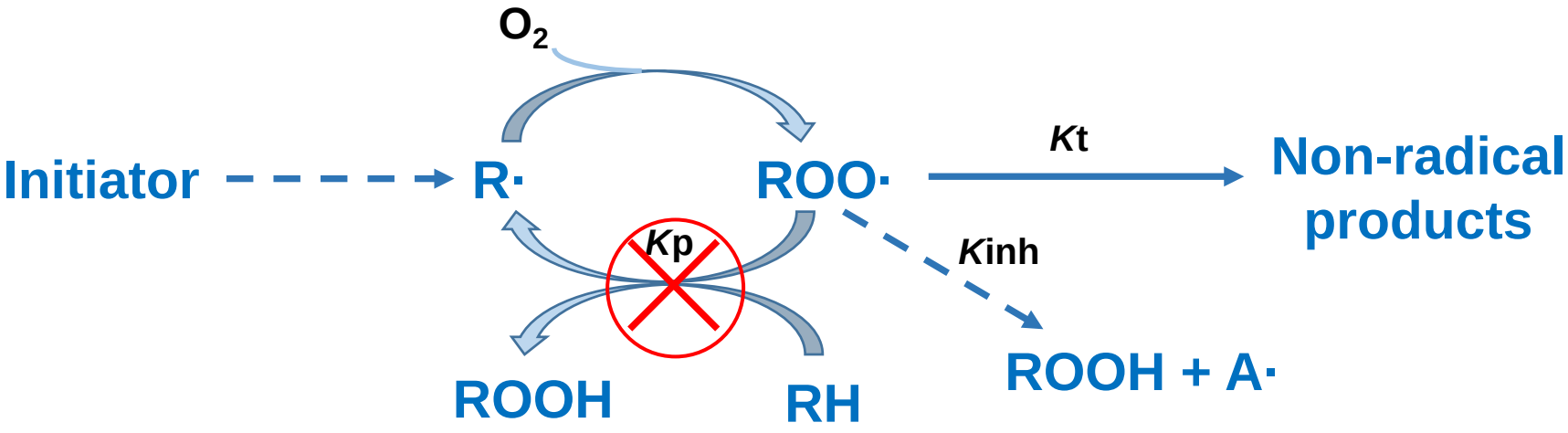
Antioxidants – Level 1



- **Preventive antioxidants**
  - **Suppress the formation of free radicals; SODs, CAT, GSH-Px**

Amorati et al., 2013; Lobo et al., 2016

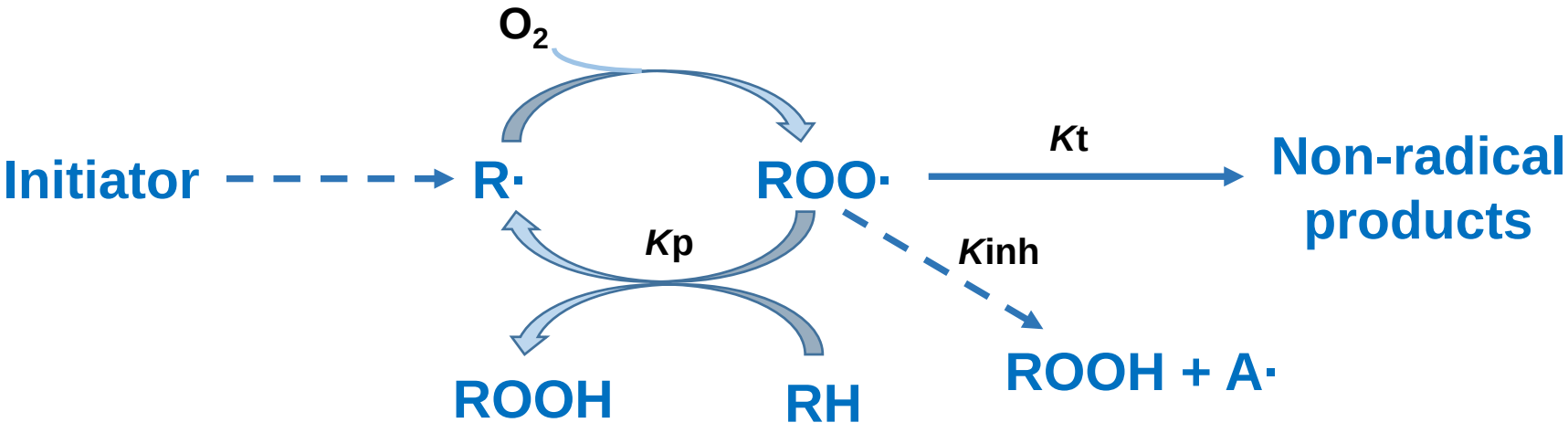
Antioxidants – Level 2



- **Radical-scavenging antioxidants**
  - **Suppress chain initiation and/or break the chain propagation reactions, such as vitamin C and E**

Amorati et al., 2013; Lobo et al., 2016

Antioxidants –Level 3



- **Repair antioxidants**
  - **Remove oxidatively modified proteins, such as proteolytic enzymes**

Amorati et al., 2013; Lobo et al., 2016

Type of antioxidants

- **Endogenous antioxidants**
  - **Enzymatic antioxidants (SODs, CAT, GSH-Px)**
  - **Non-enzymatic antioxidants (ascorbic acid, Glutathione, melatonin, vitamin E, uric acid)**
- **Exogenous antioxidants**
  - **butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), Se and vitamin E**
  - **Plant extracts**

Plant extracts & antioxidant effects

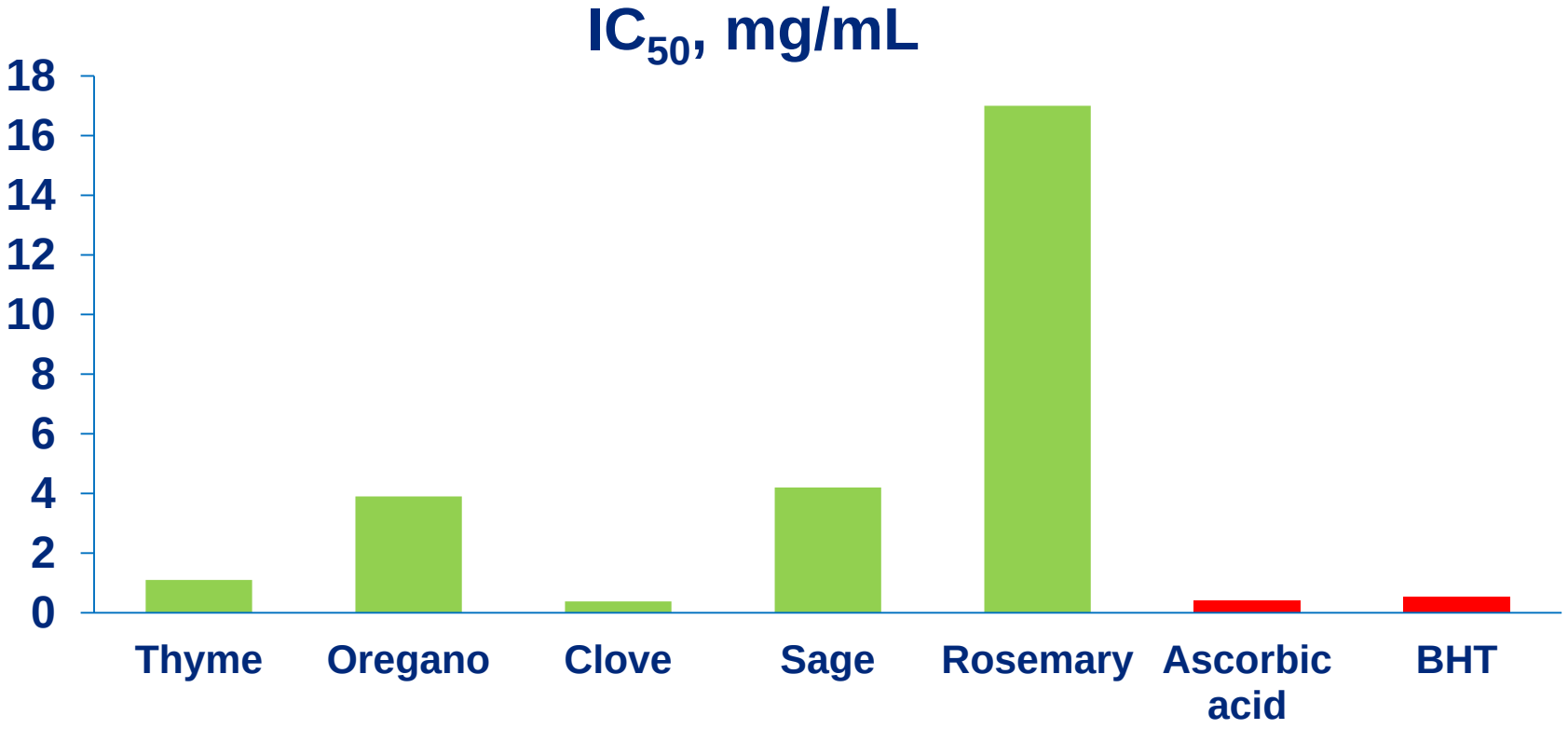
- **Phenolic compounds (carvacrol, thymol, eugenol, etc.)**
- **Other volatile constituents (e.g., sulfur-containing components of garlic or onions)**

Total phenols content

Plant extracts	Total phenols Gallic acid equivalent (µg/mL)
Clove	899
Thyme	784
Oregano	764
Rosemary	225
Sage	123

Viuda-Martos et al., 2009

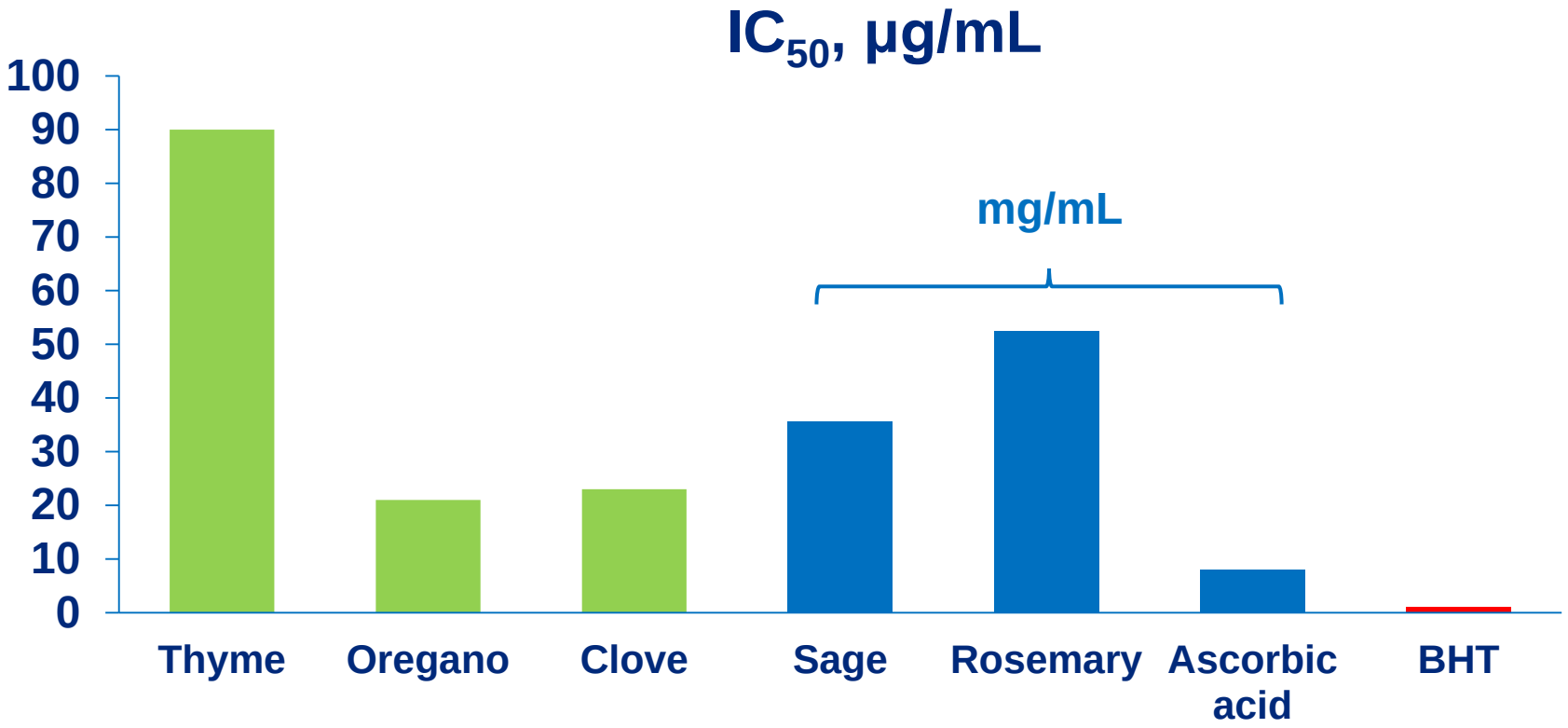
Antioxidant activities
DPPH method



IC_{50}: concentration (mg/mL) for a 50% inhibition

Viuda-Martos et al., 2009

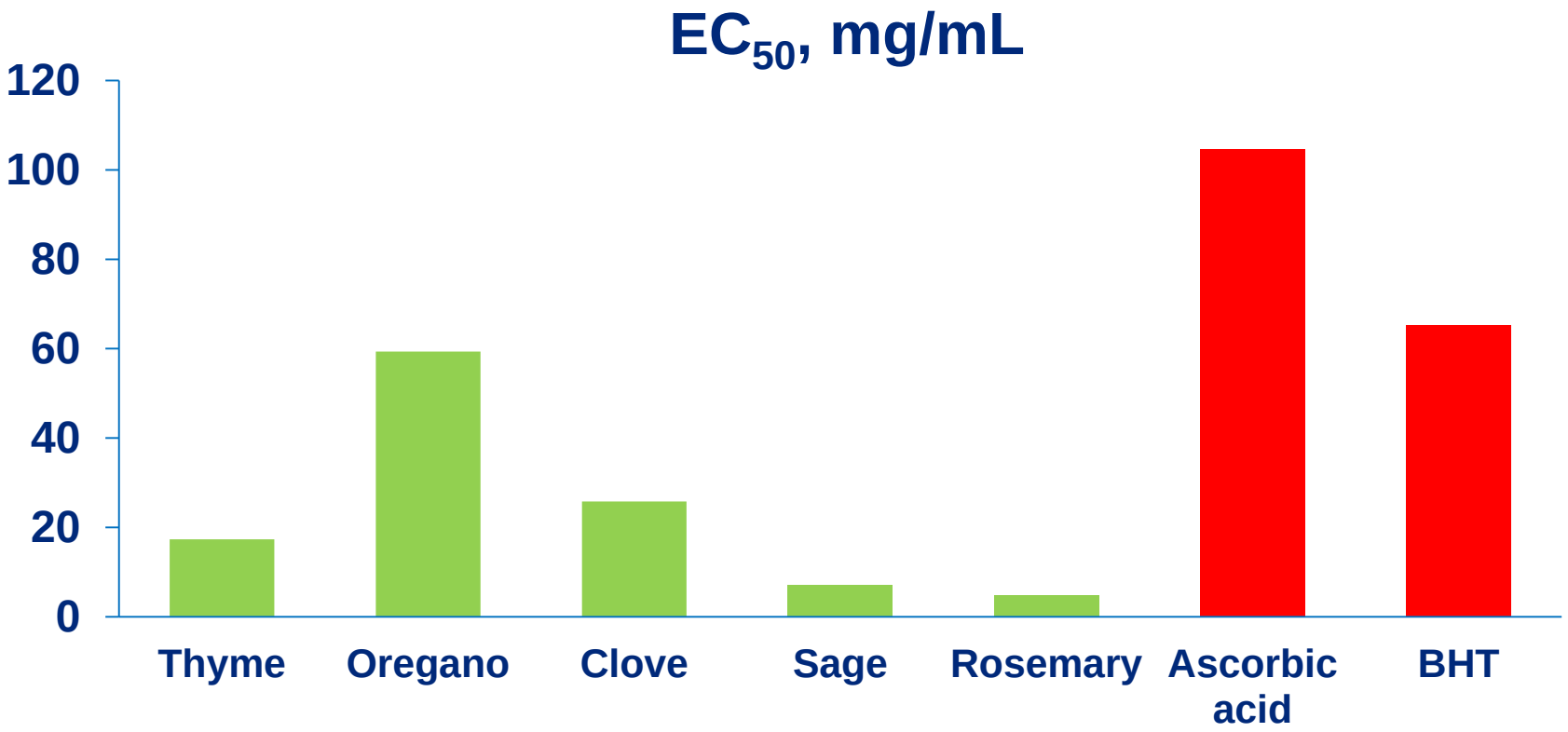
Antioxidant activities
TBARS assay



IC_{50}: concentration (µg/mL) for a 50% inhibition

Viuda-Martos et al., 2009

Antioxidant activities
Ferrous ion-chelating (FIC) assay

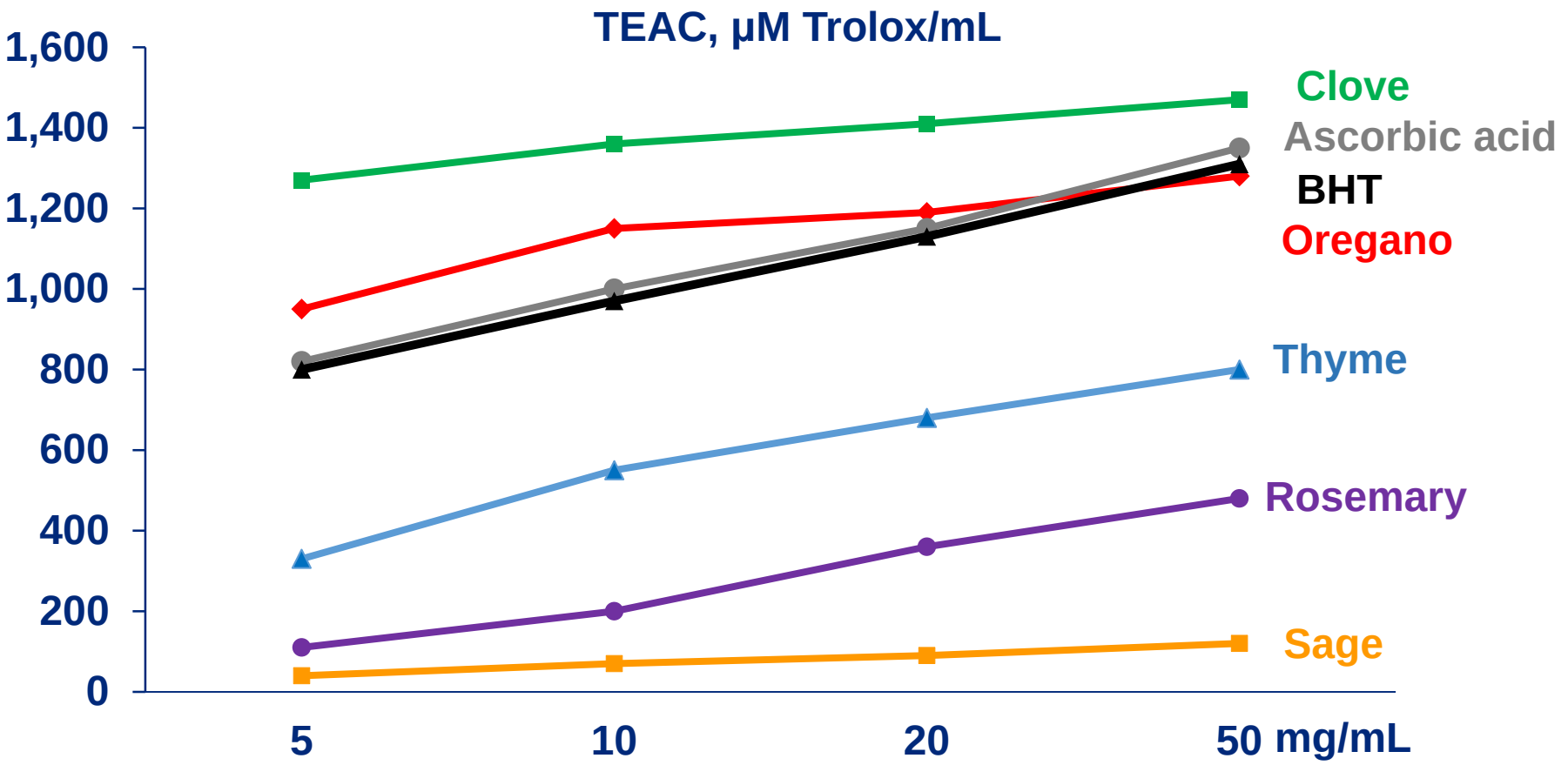


EC_{50}: concentration (μg/mL) for a 50% chelating effect

Viuda-Martos et al., 2009

Antioxidant activities

FRAP (Ferric reducing antioxidant power) assay



TEAC: Trolox equivalent antioxidant capacity

Viuda-Martos et al., 2009

In vitro antioxidant effects summary

- **DPPH: Clove > Thyme > Oregano > Sage > Rosemary**
- **TBARS: Oregano > Clove > Thyme > Sage > Rosemary**
- **FRAP: Clove > Oregano > Thyme > Rosemary > Sage**
- **FIC: Rosemary > Sage > Thyme > Clove > Oregano**
- **Results obtained from different in vitro methods are variable**

Viuda-Martos et al., 2009

In vitro methods for antioxidant activities summary

- **Chemical-based antioxidant activity**
- **Pros: simple and fast**
- **Cons: not consider certain parameters in complex cell environments; mechanisms of antioxidants are not only by scavenging free radicals**

Lipid peroxidation assay

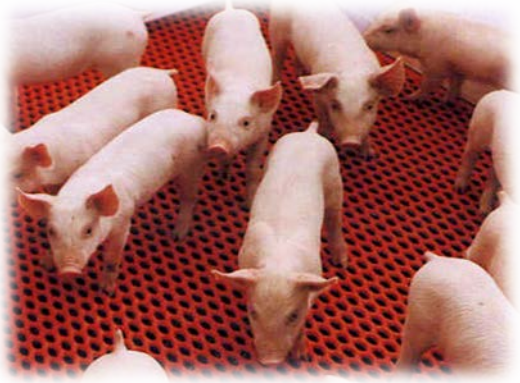
- **Lipid peroxidation: the oxidative degradation of lipids. In this process, free radicals take electrons from the lipids, resulting in cell damage**
- **Sensitively detect the concentration of MDA present in a variety of samples (liver and brain)**
- **One of most widely accepted assays for oxidative damage**

Cellular antioxidant activity

- **Very attractive testing method to support antioxidant research prior to animal studies**
- **Shows high physiological quality in antioxidant measurements**
- **Applied to product extracts, foods, dietary supplements**
- **Cheaper compared with animal studies**

In vivo animal trials

- **Highly recommended!**
  - **Dose effects**
  - **Mechanisms of action**
  - **Different stress conditions**



Overall summary

- **Reducing oxidative stress should be taken into account to promote pig health and production, especially in weaning stage**
- **Anti-inflammatory effects of plant extracts have been confirmed both in vitro and in vivo**
- **Plant extracts are potential antioxidants that can be added to animal feed**

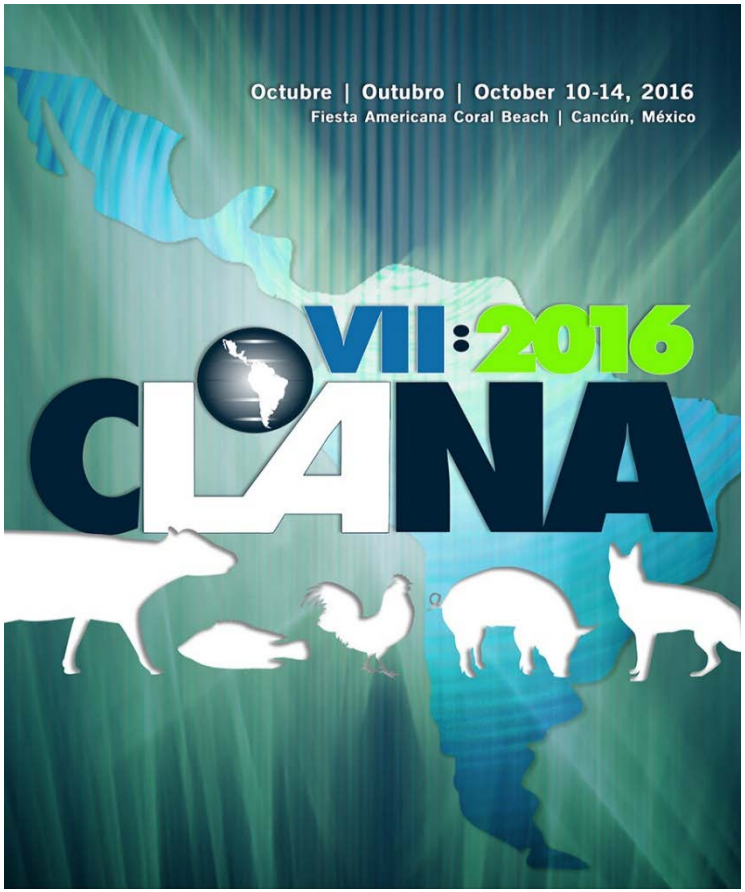
Future research

- **Correlations between chemical-based methods, lipid peroxidation assay, and cellular antioxidant assay should be conducted to provide theoretical guidance in rationally screening anti-oxidant components**
- **More research are needed to verify the antioxidant activities of plant extracts supplemented to animal feed**

Acknowledgements

- CLANA
- Pancosma

pancosma
just makes sense



Comparative Animal Nutrition & Physiology Laboratory

http://animalnutr-ansci.faculty.ucdavis.edu/