The use of polyphenols
to obtain a broad antioxidant effectiveness

provimi
shaping tomorrow's nutrition

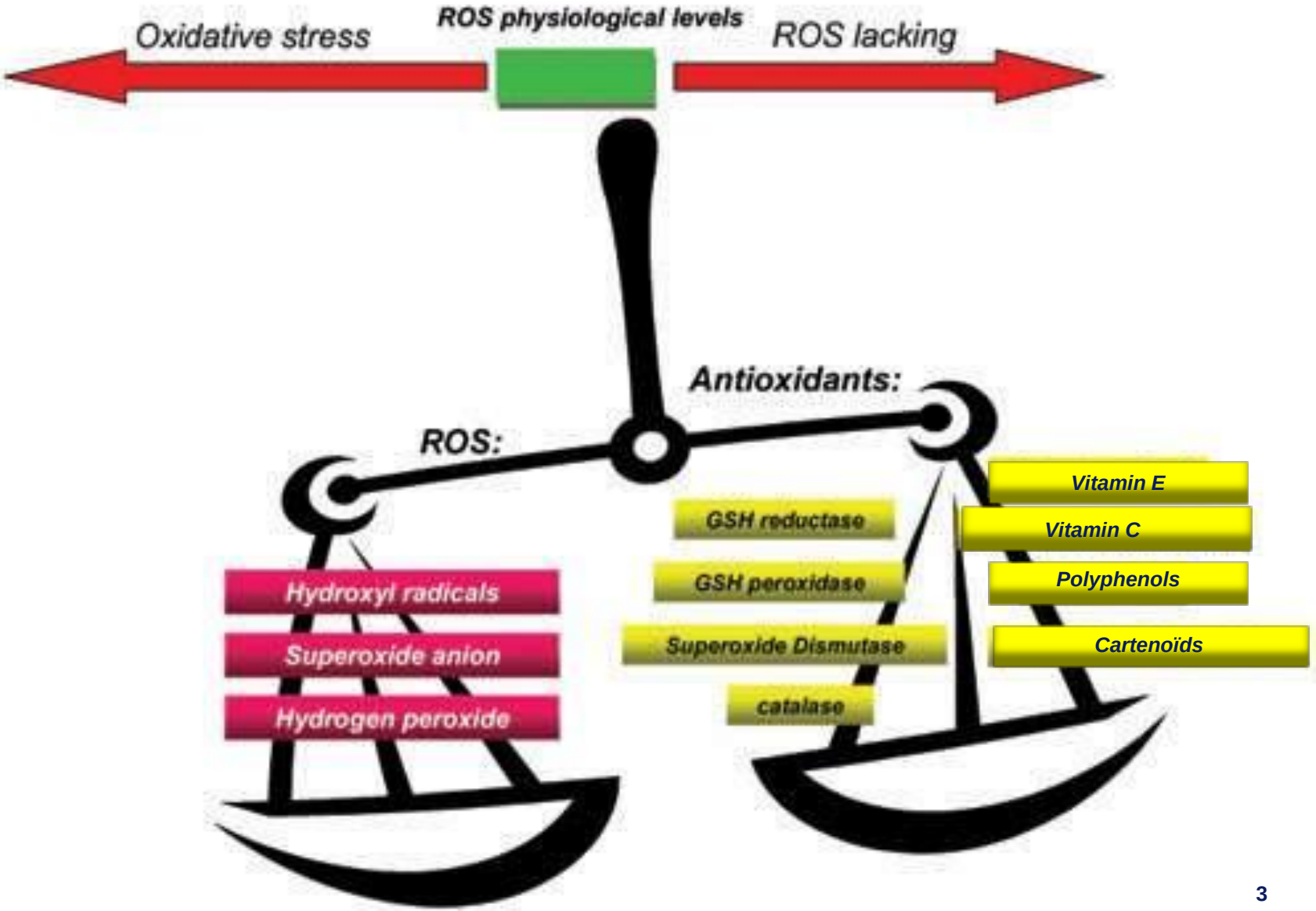
Overview presentation

- **Oxidative Stress**
  → causes and consequences
- **Antioxidants**
- **Alternative antioxidants: Polyphenols**
- **Polyphenols in poultry and swine diets**

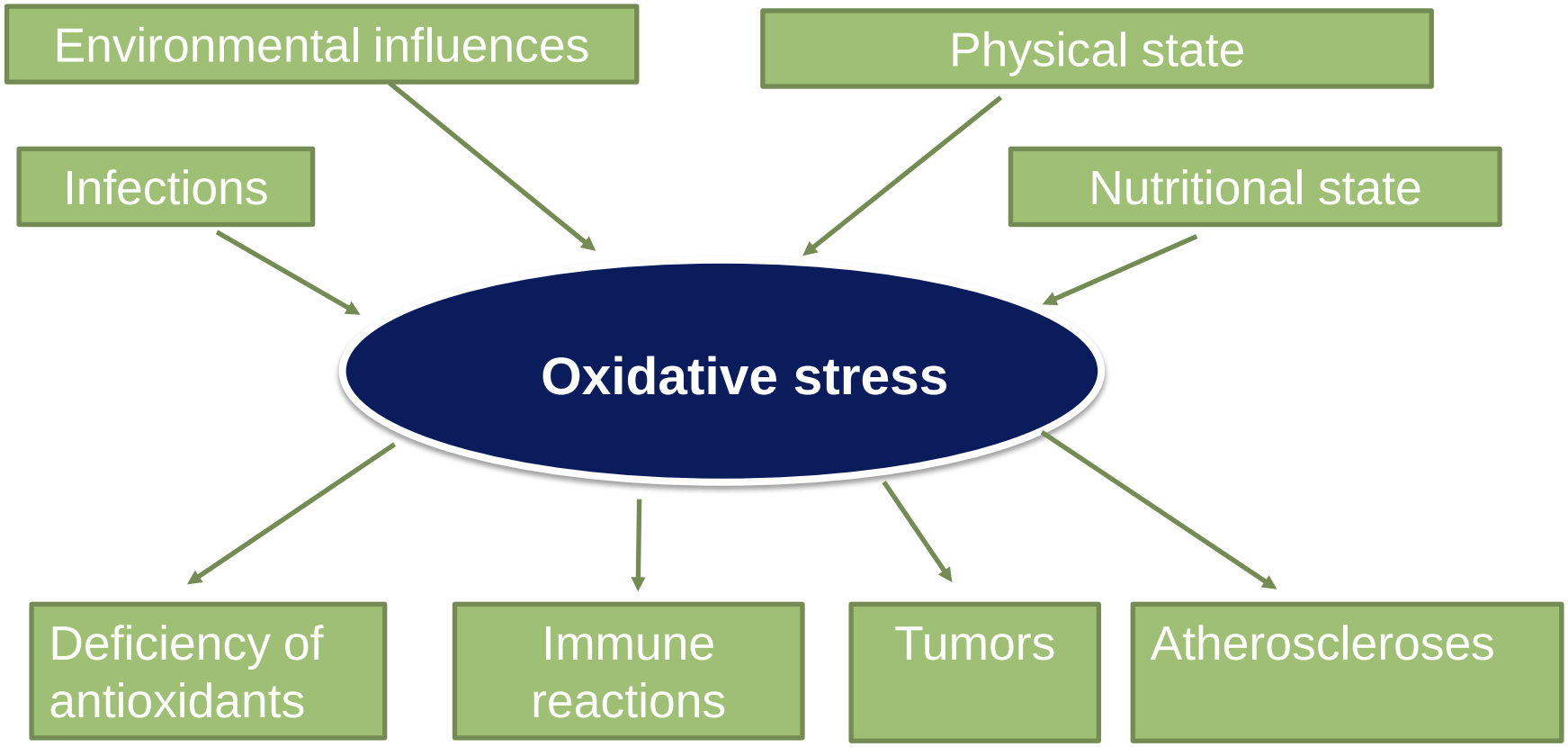
General introduction
Oxidative stress: free oxygen radicals

- **Arise naturally in metabolism of different nutrients (+/- 5% of these processes).**
- **Antagonists of these free radicals are antioxidants. The right balance between these two prevents cell damage.**
- **If free radicals out number the antioxidants an oxidative stress is occurring.**

Oxidative stress balance



Causes of oxidative stress



Nutritional causes of oxidative stress

- **Unbalanced nutrition**
- **Oxidation or high inclusion of (poly-)unsaturated fatty acids in feed**
- **Poisoning of feed by**
  - → Using raw materials with fungicides and mycotoxins
  - → High levels of heavy metals (cadmium, lead, mercury)
- **Deficiency of antioxidants e.g. vitamin E and vitamin C**
  - → precursors like e.g. selenium, beta-carotene, sulphur, zinc, cupper and manganese

Consequences of oxidative stress

- **Immune suppression**
  - → Loss of macrophages, phagocytes etc.
  - → Decreased formation of anti-bodies
  - → Increased sensitivity for infections
- **Growth inhibition, fertility disorders**
  - → Fertility depression
  - → Lower vitality at hatch
- **Liver damage**
  - → More damage by myco-/endotoxins
- **Muscle degeneration**
  - → Meat quality

Oxidative stress affects the functionality of animals as a whole

Overview presentation

- **Oxidative Stress**
  → causes and consequences
- **Antioxidants**
- **Alternative antioxidants: Polyphenols**
- **Polyphenols in poultry and swine diets**

Antioxidants

Endogeneous

- Enzymes
  - e.g. SOD, GSH-Px
- Metal sequestration proteins
  - e.g. transferrins, albumin
- Non-protein
  - e.g. coenzyme Q, glutathione

DIETARY

Synthetic

- BHT
- BHA
- TBHQ
- Propyl-gallate
- Etoxyquine

Natural

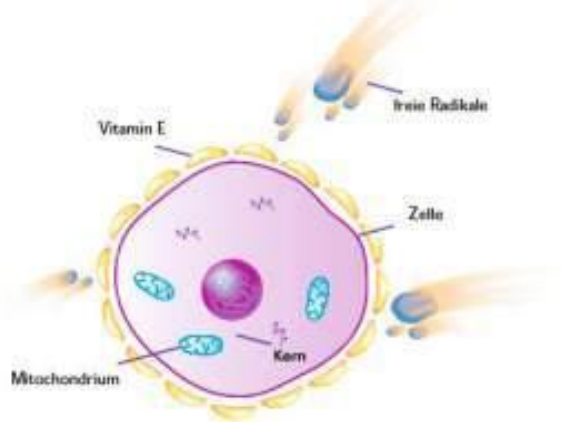
-Vitamins
  - e.g. Vit. E, Vit. C
- Minerals
  - e.g. Cu, Zn
- Carotenoids
- Polyphenols
  - e.g. phenolic acids, flavonoïds, procyanidins

Source: N. Hermans, 2008.

Most commonly known antioxidants

- **Vitamin E**
  - → Important fat soluble antioxidant in body cells
  - → Part of cell membranes.
  - → Known protection of (poly-)unsaturated fatty acids, enzymes and transport proteins
  - → Important in vivo antioxidant preventing cell damage by free radicals

- **Vitamin C**
  - → Immune system
  - → Connective tissue, bone mineralization
  - → Hormone production and fertility
  - → Iron metabolism



- **Selenium**
  - → Not an antioxidant itself, but part of the glutathione peroxidise enzyme
  - → This enzyme protects against cell damage by free radicals
  - → Assists in removal of peroxides out of body cells, formed during oxidation

Combination of different antioxidants is effective

- **All anti-oxidants together are an umbrella to protect for free radicals. They are working as a team. They have to play together; only one kind doesn't work.**

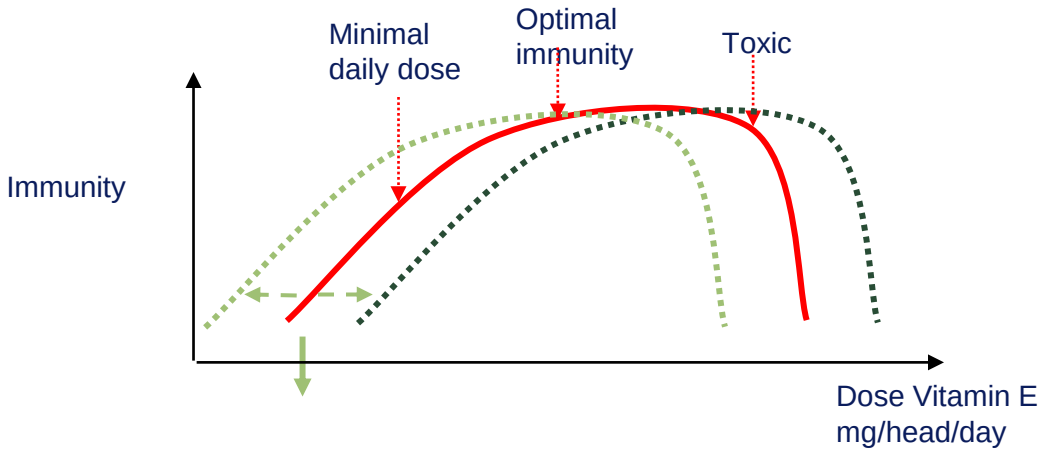
  (Source: P. Surai, European Poultry Congress of WPSA, Verona (2006))

- **A diet with a high content and wide variety of antioxidants appears to offer some health advantage. While a narrow range of antioxidant are of unproven efficacy and of possible harm.**

  (Source: T.I. Mbata, Internet Journal of Food Safety V (7): 29-33)

Overdosing vitamin E makes it act as a pro-oxidant resulting in negative effects

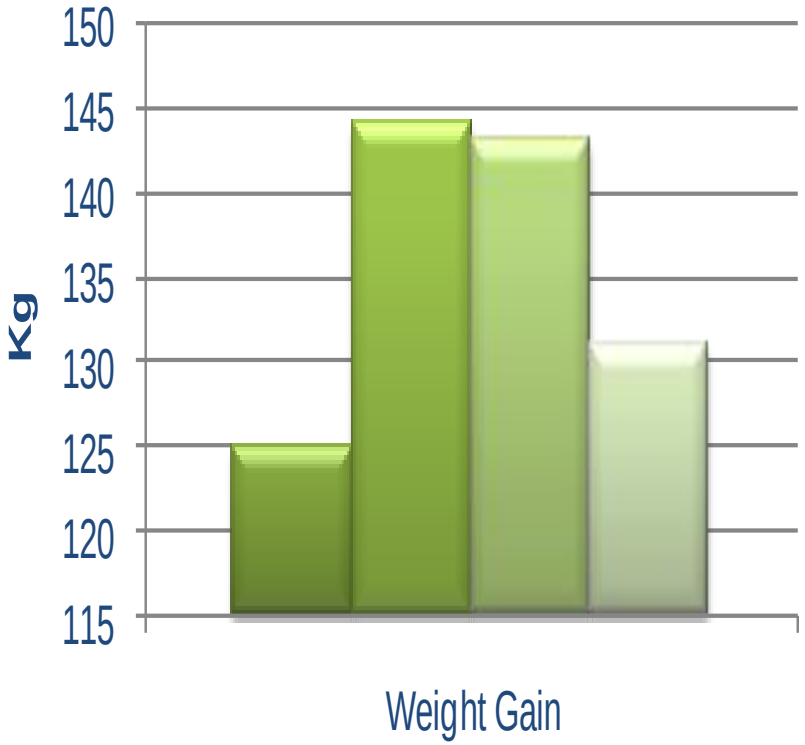
- **In humans: meta-analysis was done with conclusion: Regular administration of high-dose vitamin E supplements may be associated with increased mortality. The biological mechanism for this effect is uncertain (Source: Pearson et al.)**
- **General in monogastrics**

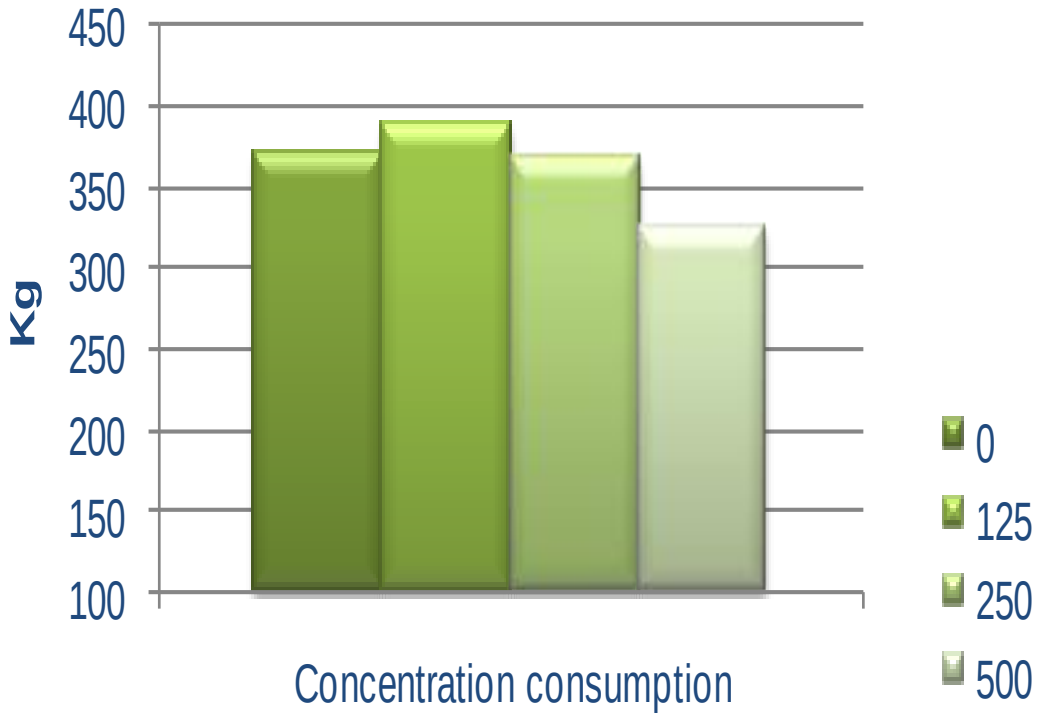


Source: K. Klasing personal communication

Example: vitamin E dose response in calves

- **Effect of supplemental vitamin E on performance**
- **32 Holstein heifer calves**
- **Four treatments (0,125, 250, or 500 IU suppl.vit E /calf/day)**





Source: P.G. Reddy, J.L. Morrill, and R.A. Frey, Journal Dairy Science 70:123-129.

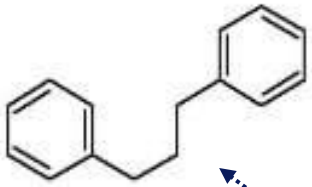
Overview presentation

- **Oxidative Stress**
  → causes and consequences
- **Antioxidants**
- **Alternative antioxidants: Polyphenols**
- **Polyphenols in poultry and swine diets**

Alternative antioxidant: Flavonoids

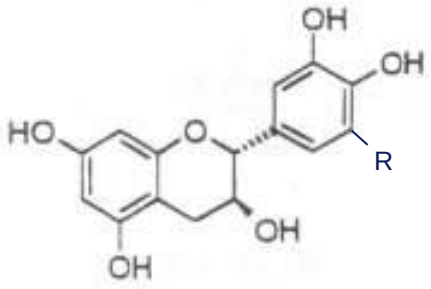
- **Polyphenol structures**
- **Present in plants**
- **More then 6.500 different variants**
- **Give plants their different colours**
- **Protect plants against damaging UV-light.**

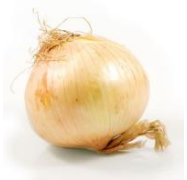
Flavonoïds: 6500 varieties



Basic structure

- Polyphenols
  - Flavonoïds
    - Flavan-3-ols
      - Catechin
      - Epicatechin
      - Other groups
    - Flavanones
    - Flavanols
    - Anthocyanidins
    - Flavones
  - Tannins
  - Lignin





Source: USDA, 2003

Flavonoïds

Source: B.H. Havsteen, Pharmacology & Therapeutics 96 (2002) 67–202.

Anthocyanidins	hydroxyl-4-dihydroflavonoles glycosides of anthocyanidines
Flavonoles & Iso-flavonoles	2-phenyl-3-hydroxy-chromones 3-phenyl-2-hydroxy-chromones
Flavones & Iso-flavones	2-phenyl-chromones 3-phenyl-chromones 3-phenyl-2-dihydro-chromones
Flavanes & Iso-flavanes	2-phenyl-3-dihydro-chromones 2-phenyl-flavanones 2-phenyl-di-hydro-benzo-g-pyranes 3-phenyl-di-hydro-g-benzo-pyranes
Flavanols & Iso-flavanols	2-phenyl-3-hydro-3-hydroxy-chromones (catechins) 9, 2-hydro-2-hydroxy-3-phenyl-chromones
Aurones	benzo-furones
Coumarins	benzo-g-pyron derivatives

Flavonoids

Flavonoids – 6 Groups

with 6.500 known variants

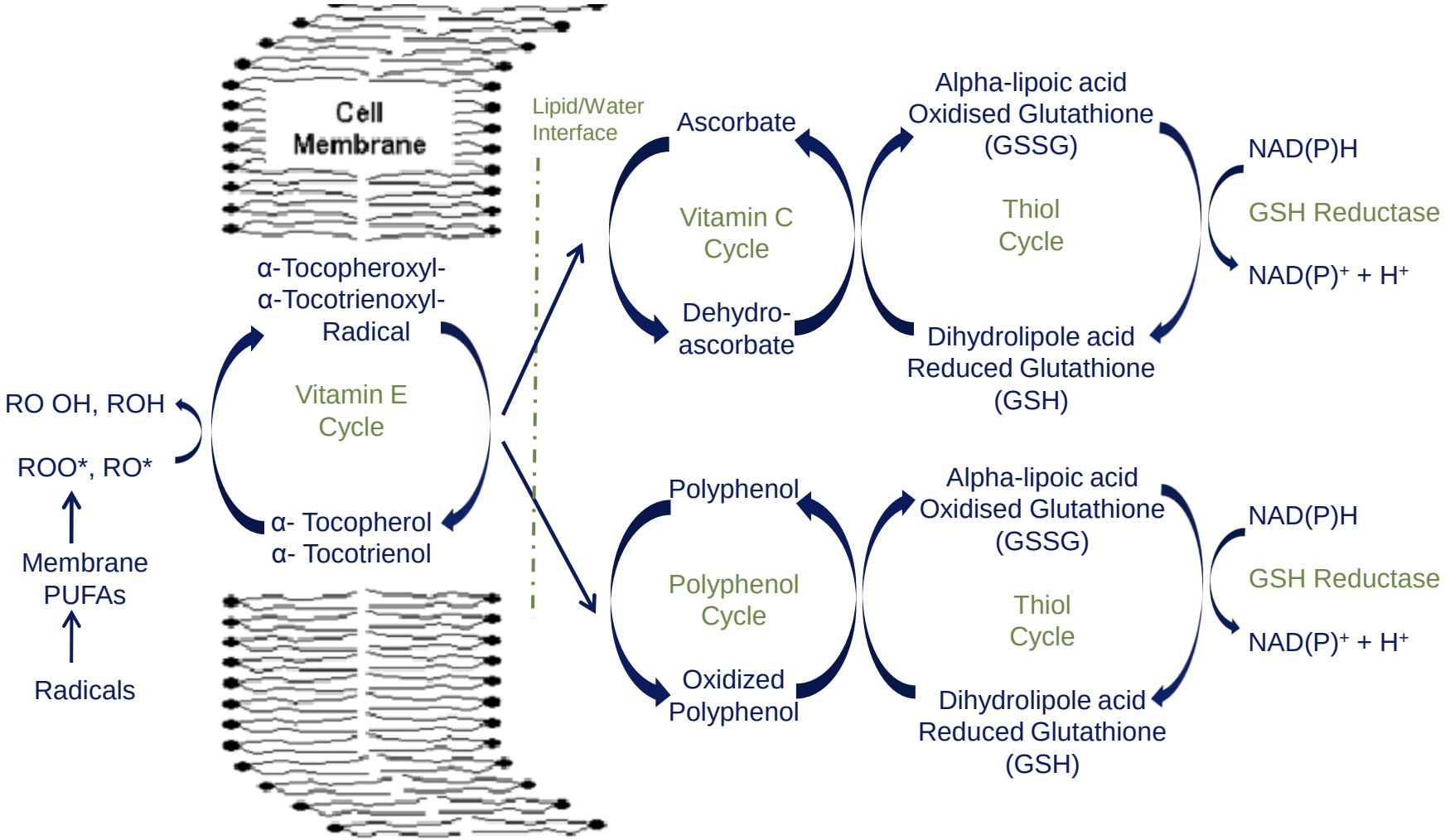
Flavone	Flavonole	Flavanone	**Flavanoid**	Isoflavonoid	Anthocyane

Content in mg/kg or mg/l (By Linseisen u.a. 1997)	**Monomeres**	
	Catechin	Epicatechin
Grape vine, ret	90	87
Wine red	110	31
Tea black	6	26
Cherry, acid	16	98
Apple	10	81
Blackberry	14	112
Chocolate	132	327
Grape kernel	2.970	1.620

Content mg/kg or mg/l (By TUM)	**Oligomers**
	Proanthocyanidins
Apple	211
Pear	0,26
Kiwi	0,05
Grape, skin	20
Cherry, acid	traces
Crataeugus monogyna	traces
Grape, kernel	17.160

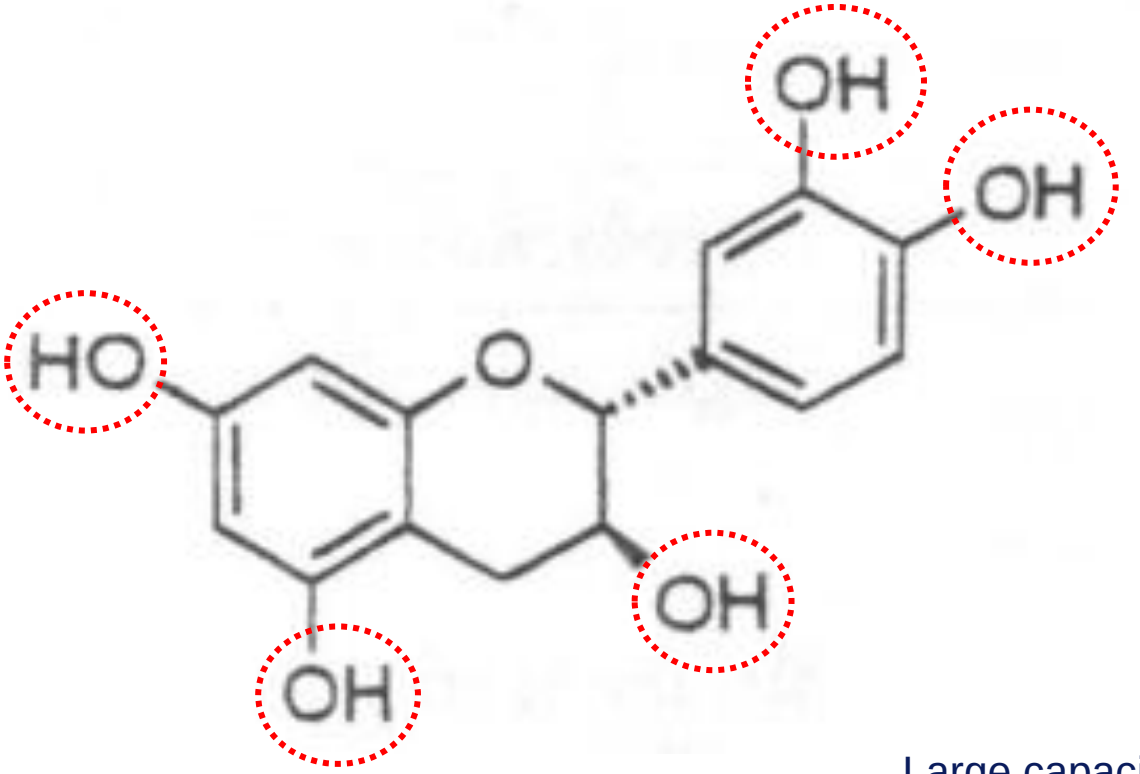
Working mechanism antioxidants in the animal

VERS = Vitamin E Recycling System



Adapted from R. Bouwstra, and Nwose et al. (2008)

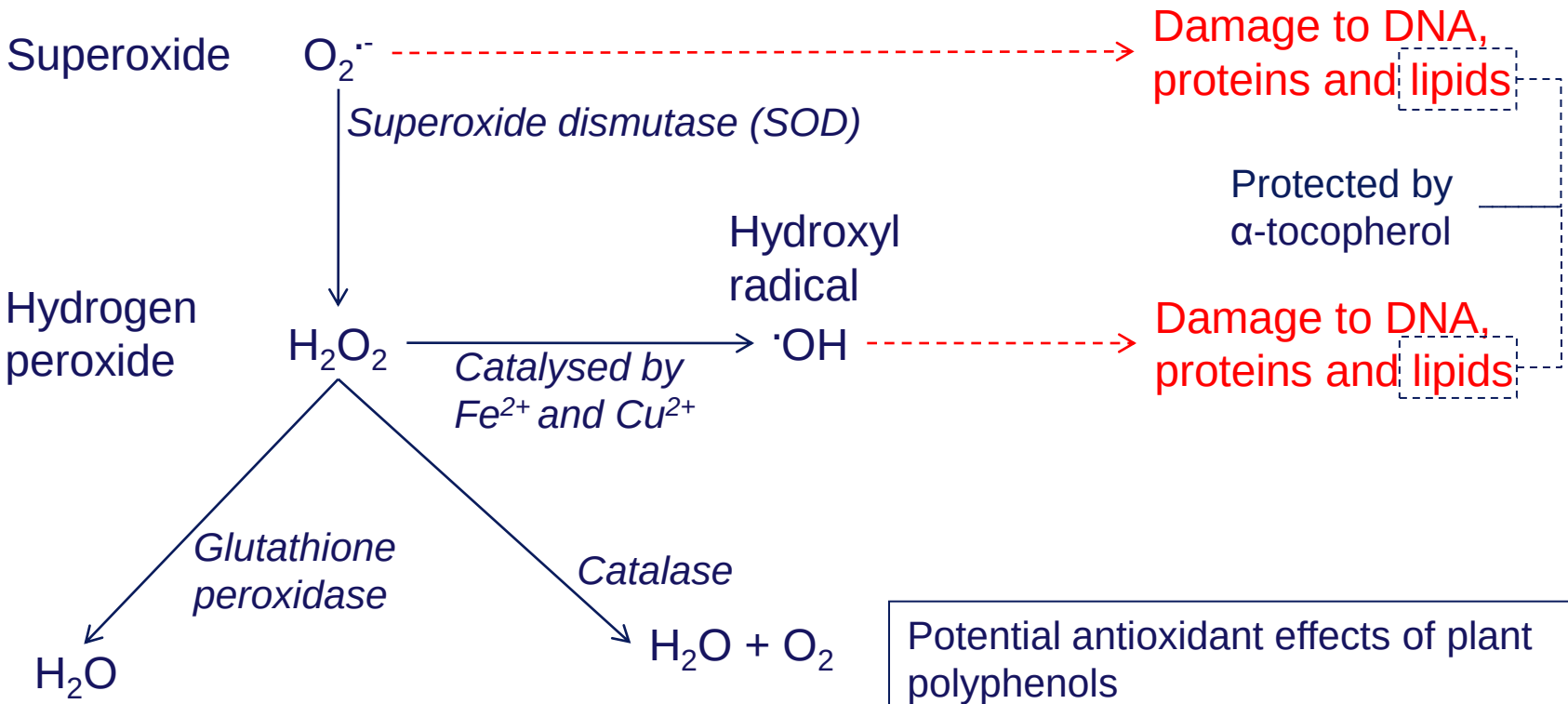
Functionality of flavonoïds



Flavonoïd type: catechin

Large capacity to give away unpaired electrons
=
Large anti-oxidant capacity

What's the relevance of plant polyphenols?



Potential antioxidant effects of plant polyphenols

- direct scavenging of reactive oxygen species
- stimulation of enzymic antioxidants
- chelation of metal ions
- re-generation of oxidised α-tocopherol

Relative antioxidant capacity of different extracts

Table 1. Polyphenol content and reducing potential of plant extracts obtained from rosemary (RO), Grape (GP), citrus (CI), marigold (MA)

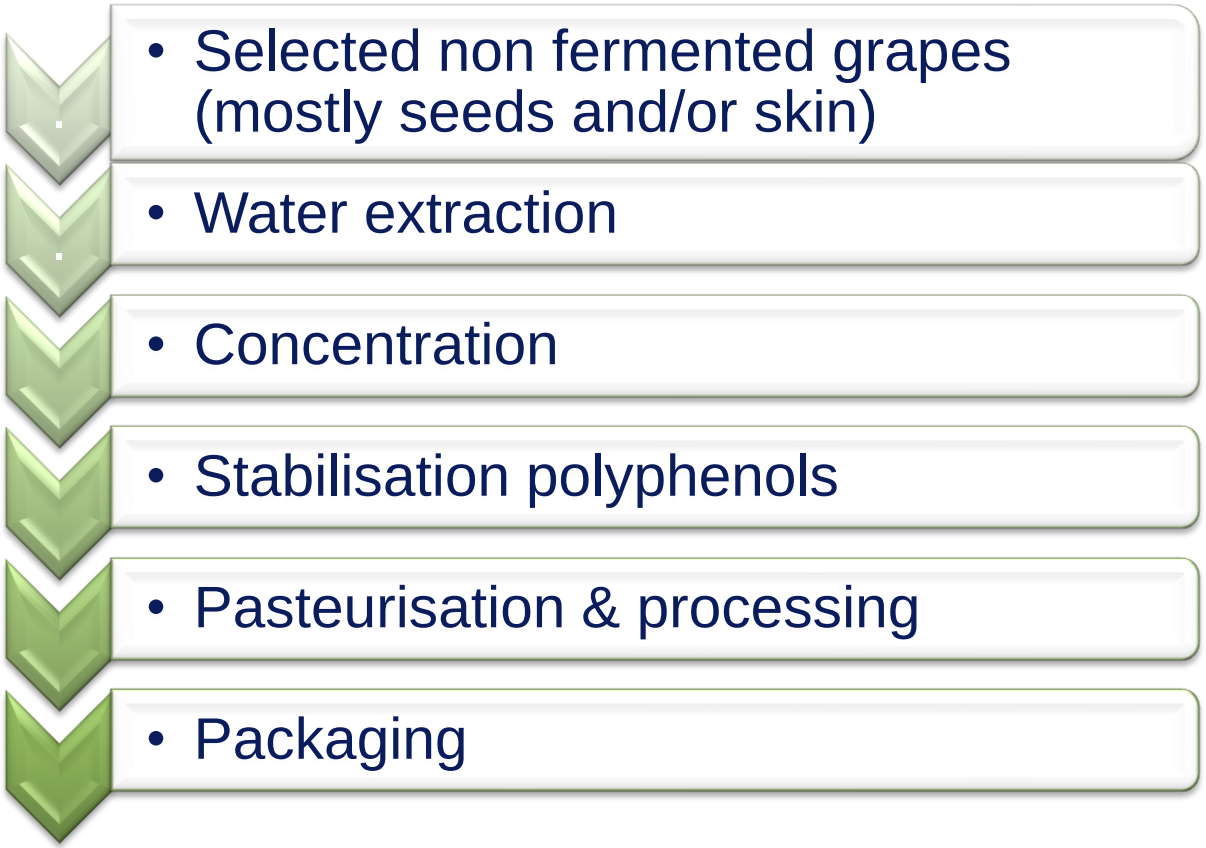
	Plant extracts			
	RO	GP	CI	MA
Polyphenol content*	16	651	213	77
Reducing potential[1]	469	6630	212	962

* Gallic acid equivalent, mg/g DM.

[1] Trolox equivalent antioxidant capacity, umol/g DM

Source: C. Glandine et.al. Br. J. Nutr. 98: 691-701

General production process of polyphenolic grape products

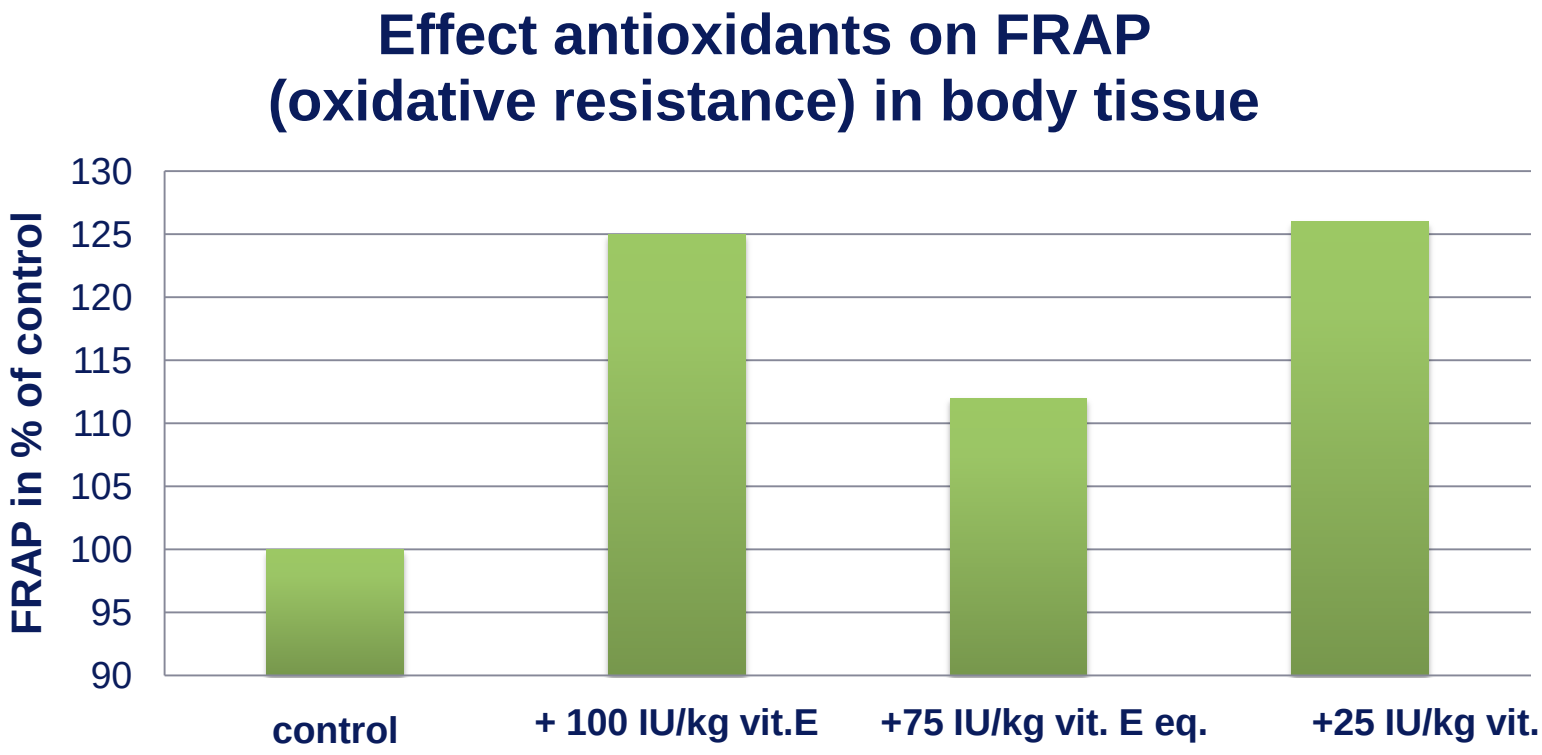


All steps in the production process are important to produce a constant product with high bio-availability

Overview presentation

- **Oxidative Stress**
  → causes and consequences
- **Antioxidants**
- **Alternative antioxidants: Polyphenols**
- **Polyphenols in poultry and swine diets**

Antioxidant trial in Provimi Broiler Bio-Assay facility



- FRAP = Ferric Reducing Antioxidant Power
  = measurement of oxidative resistance

Source: Provimi Research, The Netherlands

Antioxidant trial in Provimi Broiler Unit

- **Male Ross 308 broilers**

- **42 days on test diet**
  - → Starter: 0-14 days of age
  - → Grower: 14-35 days of age
  - → Finisher: 35-42 days of age

- **120 birds per treatment**
  - → 6 cages (=replicate)
  - → 20 birds per cage

- **Measurements**
  - → Body weight: 14, 35 and 42 days
  - → Feed intake: 14, 35 and 42 days
  - → Feed conversion ratio: kg feed consumed / kg weight gain

Source: Provimi Research, The Netherlands

Comparison of technical results of high vitamin E versus partly replacement of vitamin E by polyphenols

	100 ppm Vit E	10 ppm Vit E + 90 ppm Vit E eq of polyphenols	LSD
Mortality	3.3	3.3	-
Gain 0-14	532	531	18
Gain 0-35	2498[b]	2592[a]	73
Gain 0-42	3149	3200	96
FI 0-14	596	596	20
FI 0-35	3661	3750	93
FI 0-42	4790	4877	121
FCR 0-14	1.122	1.122	0.011
FCR 0-35	1.466	1.447	0.023
FCR 0-42	1.522	1.524	0.030
EPI	482	489	24

Source: Provimi Research, The Netherlands

Effect of polyphenol products on broiler performance

Broiler trial in France

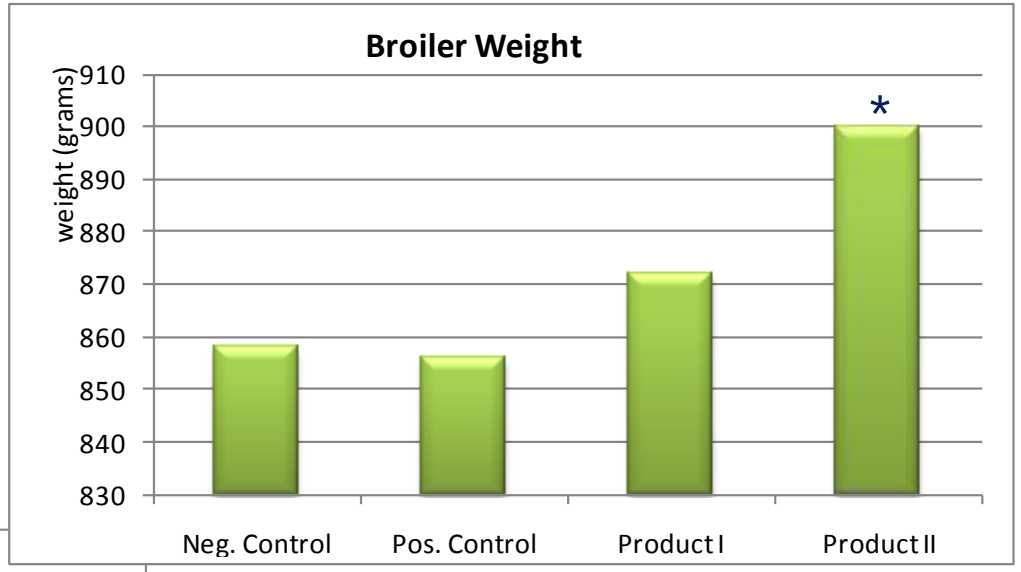
- **180 broilers, 21 days of age**

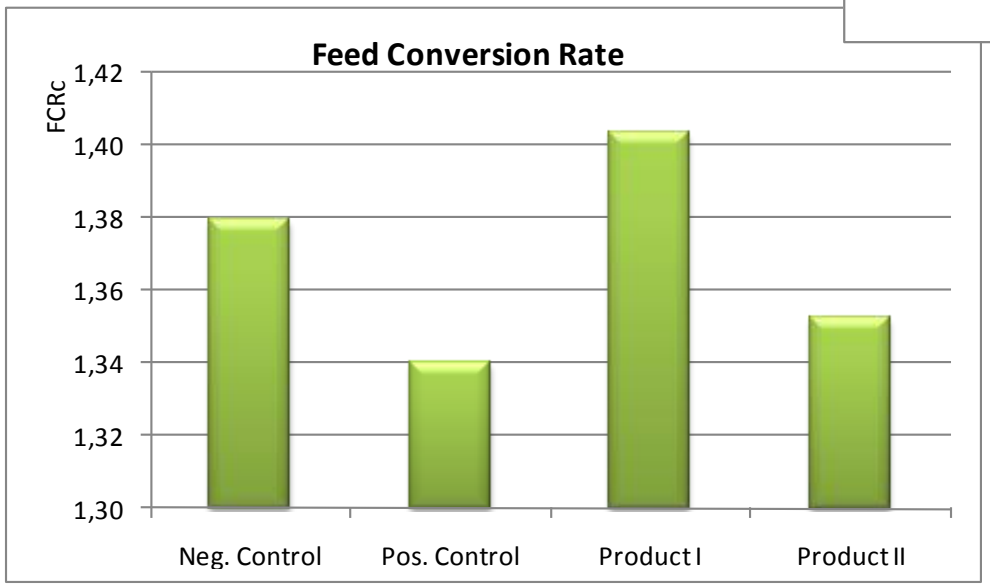
- **Treatments**
  - → Control 20 ppm Vit. E
  - → Control + 100 ppm Vit. E
  - → Control + 100 ppm Vit. E eq. of a polyphenol product I*
  - → Control + 100 ppm Vit. E eq. of a polyphenol product II*

*Product I and II are based on grape, with standardization on proanthocyanidins

Source: H Juin (INRA)

Effect of polyphenol products on broiler performance





Although both products have the same level of proanthocyanidins, results are different !
→ Bio-availability ?

Source: H Juin (INRA)

Effectiveness of polyphenols on the shelf-life of pig meat

Test Conditions:
Duration**: 10 weeks**

- Control**: 15 ppm of Vit E**
- Vitamin E Dose**: 200 ppm**
- Vitamin E Dose + polyphenol**:**
  100 ppm Vit E + 100 Vit E eq of polyphenols

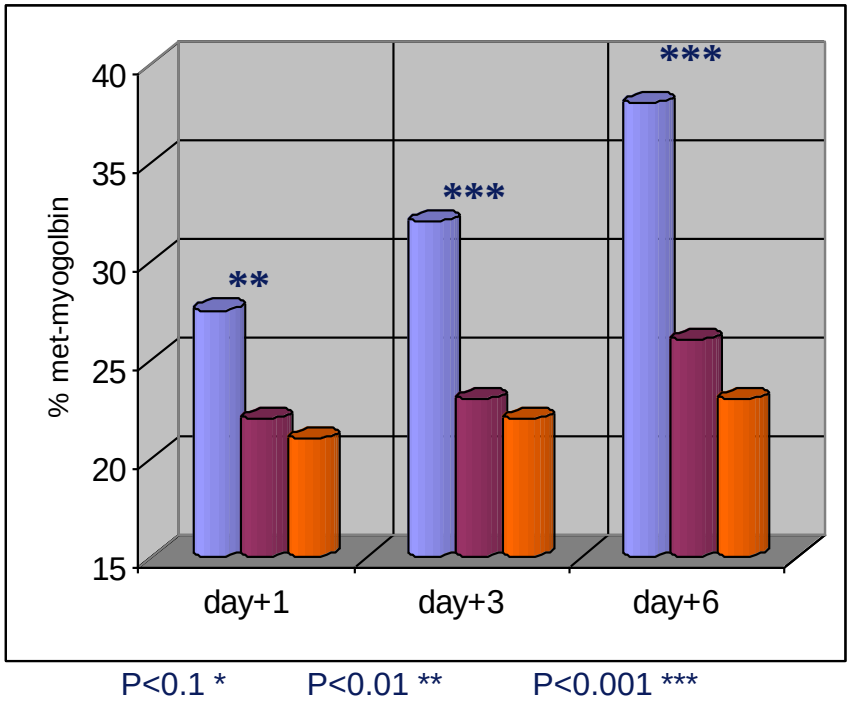
Observations:
Commercial farm conditions for pigs.
Day 0 = slaughtering

Analysis: **INRA, WPM, 2000**

Conclusion:
At day 6 meat of both positive controls still not significantly deteriorated.

Significant difference with 15 ppm vitamin E treatment

Oxidized Myoglobin
= met-myoglobin



Source: Provimi Research, France

Conclusions polyphenols in poultry & swine diets

- **There are differences in effectiveness of polyphenol products**

- **When using a good polyphenol product:**

  → Antioxidant capacity of combination polyphenols and vitamin E is at least comparable with high levels of vitamin E

  → Technical results and shelf life of meat with combination polyphenols and vitamin E are at least comparable with technical results and shelf life of meat with high vitamin E

  - Based on comparable levels of antioxidants in the diet, expressed in vitamin E equivalence

Conclusions

- **A spectrum of different antioxidants is more effective than a single antioxidant with a high inclusion rate.**
- **Polyphenols are antioxidants which can play an important role in the animal.**
- **There is a wide range of polyphenols, which have all different antioxidant capacities. Within the range of polyphenols, the flavonoïds have good antioxidant characteristics. Especially these with high levels of proanthocyanidins.**
- **Products with equal levels of polyphenols and proanthocyanidins can lead to differences in performance. This could be due to the difference in bio-availability.**


provimi
shaping tomorrow's nutrition