NUTRITIONAL STRATEGIES TO COUNTERACT OXIDATIVE STRESS: BENEFITS AND CHALLENGES

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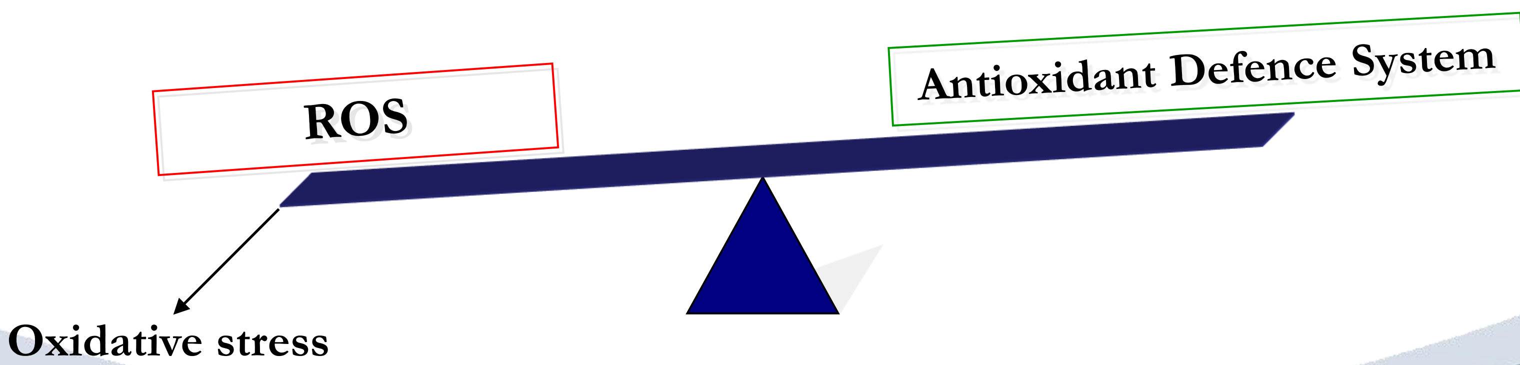
OXIDATIVE BALANCE

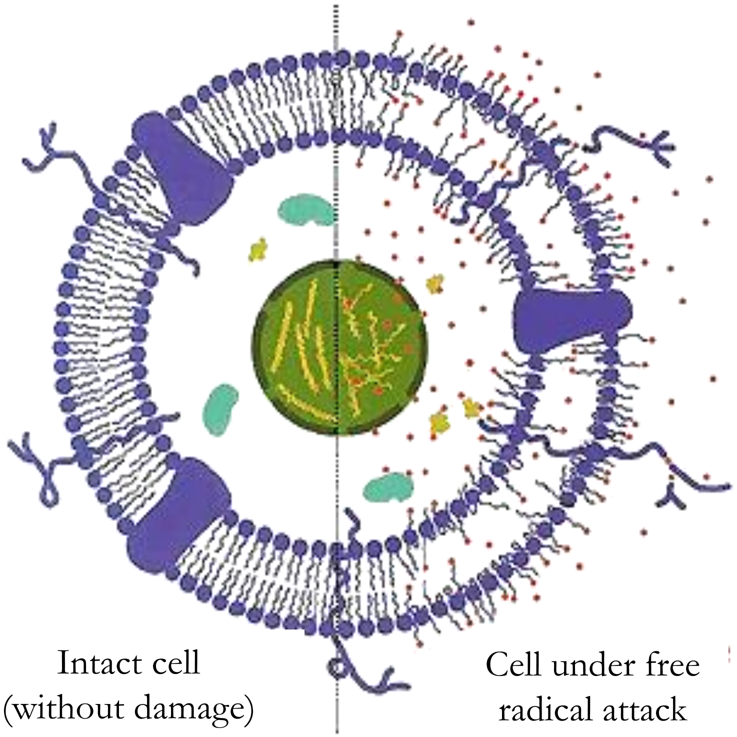
- **Reactive oxygen species (ROS)**
  - Radicals
    - Superoxide, • O^{-}_{2},
    - Hydroxyl, •OH
    - Hydroperoxyl, $H_2O•_2$
    - Alkoxyl, $RO•_2$

- **Prevention and scavenging**
  - Metalloproteins
  - Enzymes
  - Vitamins/pro-vitamins
- **Chain breaking**
  - Vitamins/pro-vitamins
  - Enzymes
- **Repair/remove**



OXIDATIVE DAMAGE

- Free Radicals are molecules containing one or more unpaired electrons
- Highly reactive at high concentrations can be dangerous to cell structures, DNA, lipids and proteins
- Cells injured by free radicals will spill free radicals onto adjacent cells generating more free radicals



FUNCTIONS OF ROS

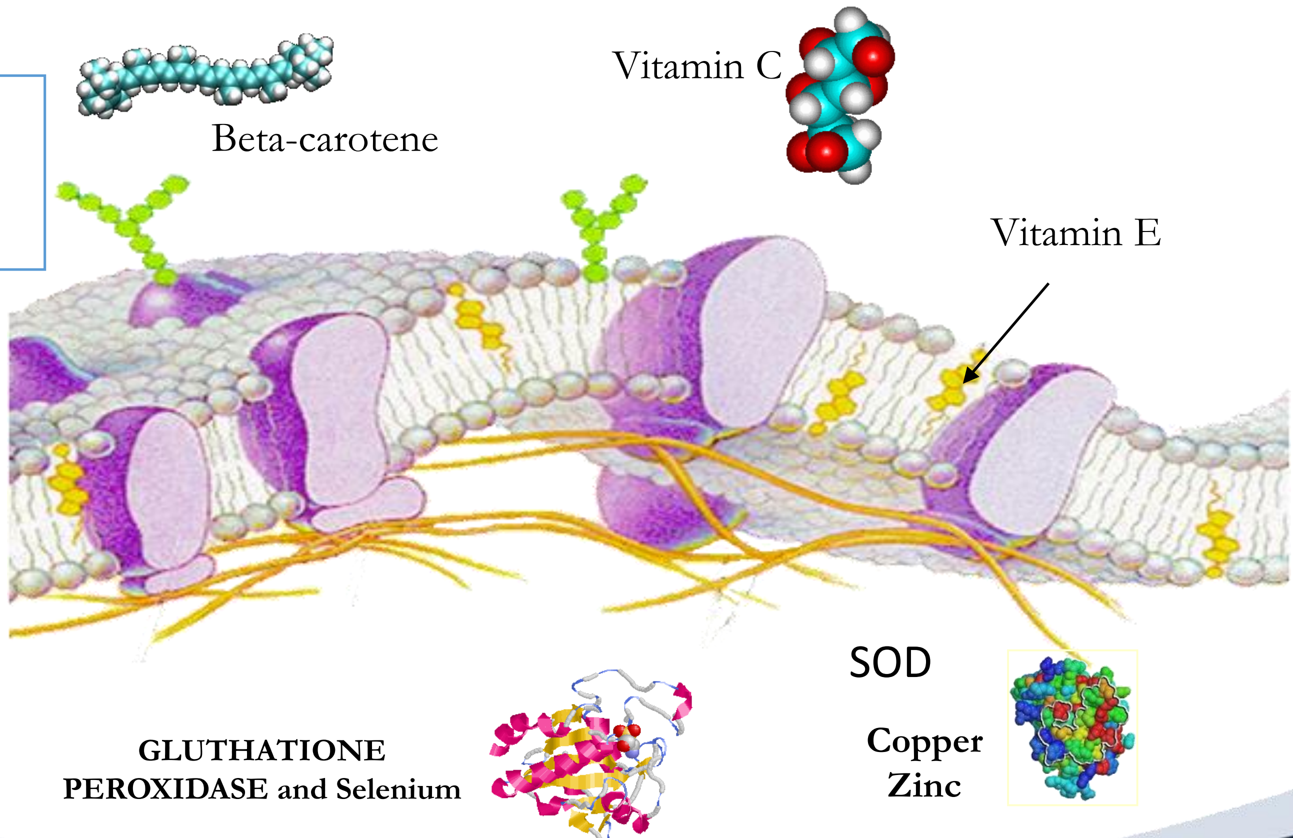
- Oxidation of polyunsaturated fatty acids
- Oxidation of amino acids
- Damage of DNA
- Inactivation of specific enzymes

- Apoptosis
- Induction of host defense genes
- Killing ability neutrophils/macrophages
- Keep antioxidant system activated

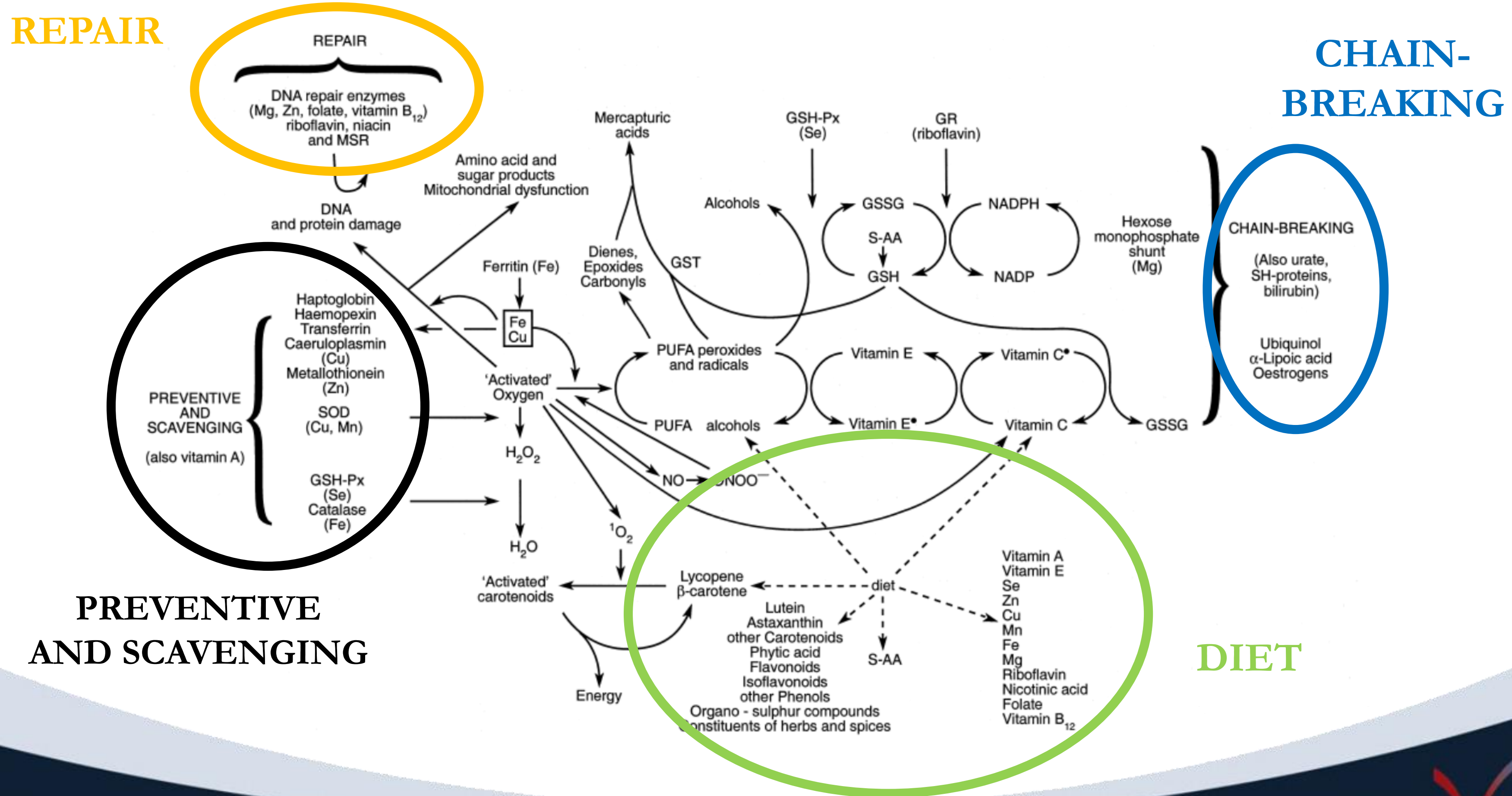
CELL ANTIOXIDANT SYSTEM IS AN INTEGRATED MACHINERY

Low molecular weight antioxidants

Enzimatic antioxidants



ANTIOXIDANT DEFENSE SYSTEM



ANTIOXIDANT DEFENSE SYSTEM

vitamin C

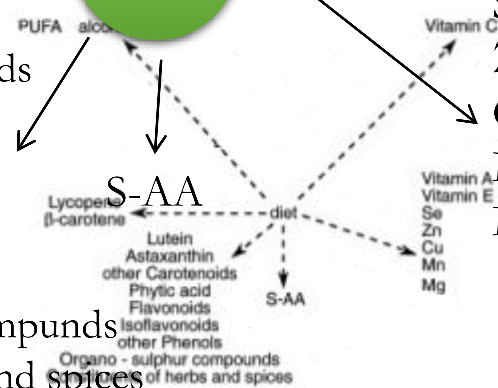
Lycopene
β-carotene

DIET

vitamin A
Vitamin E
Se
Zn
Cu
Mn
Mg

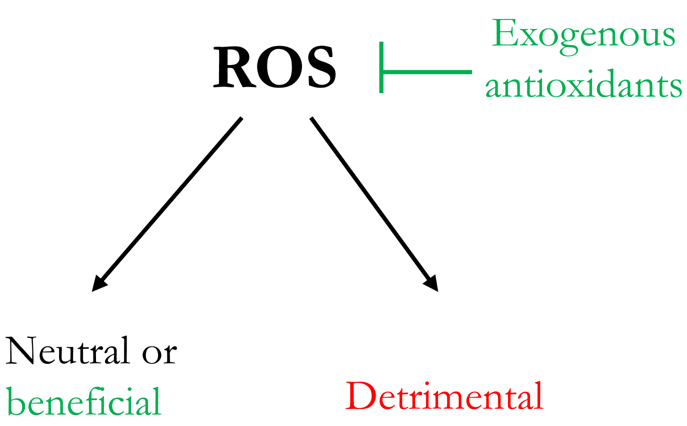
Lutein
Astaxanthin
other Caroteinoids
Phytic acid
Flavonoids
Isoflavonoids
other Phenols
Organo – sulphur compunds
Costitutents of herbs and spices

S-AA

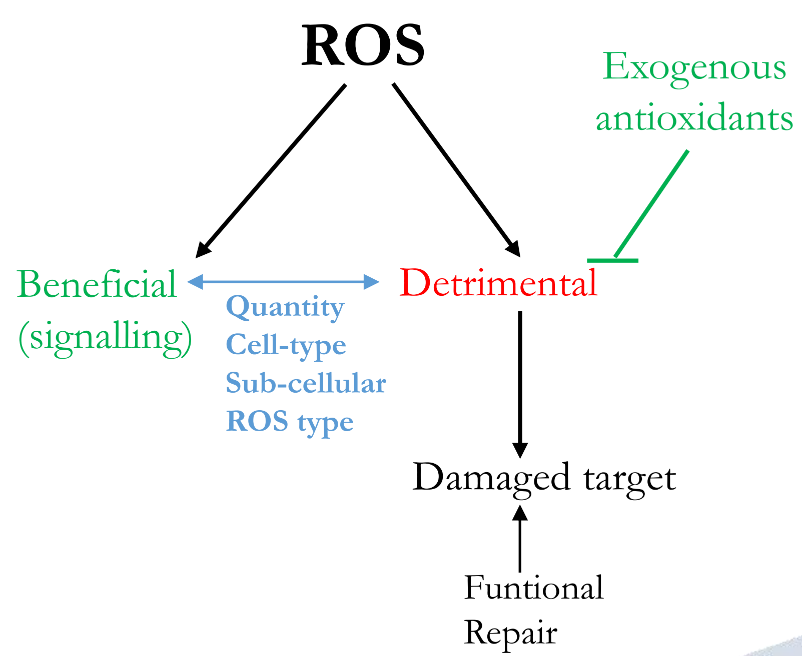


ANTIOXIDANT'S MECHANISMS

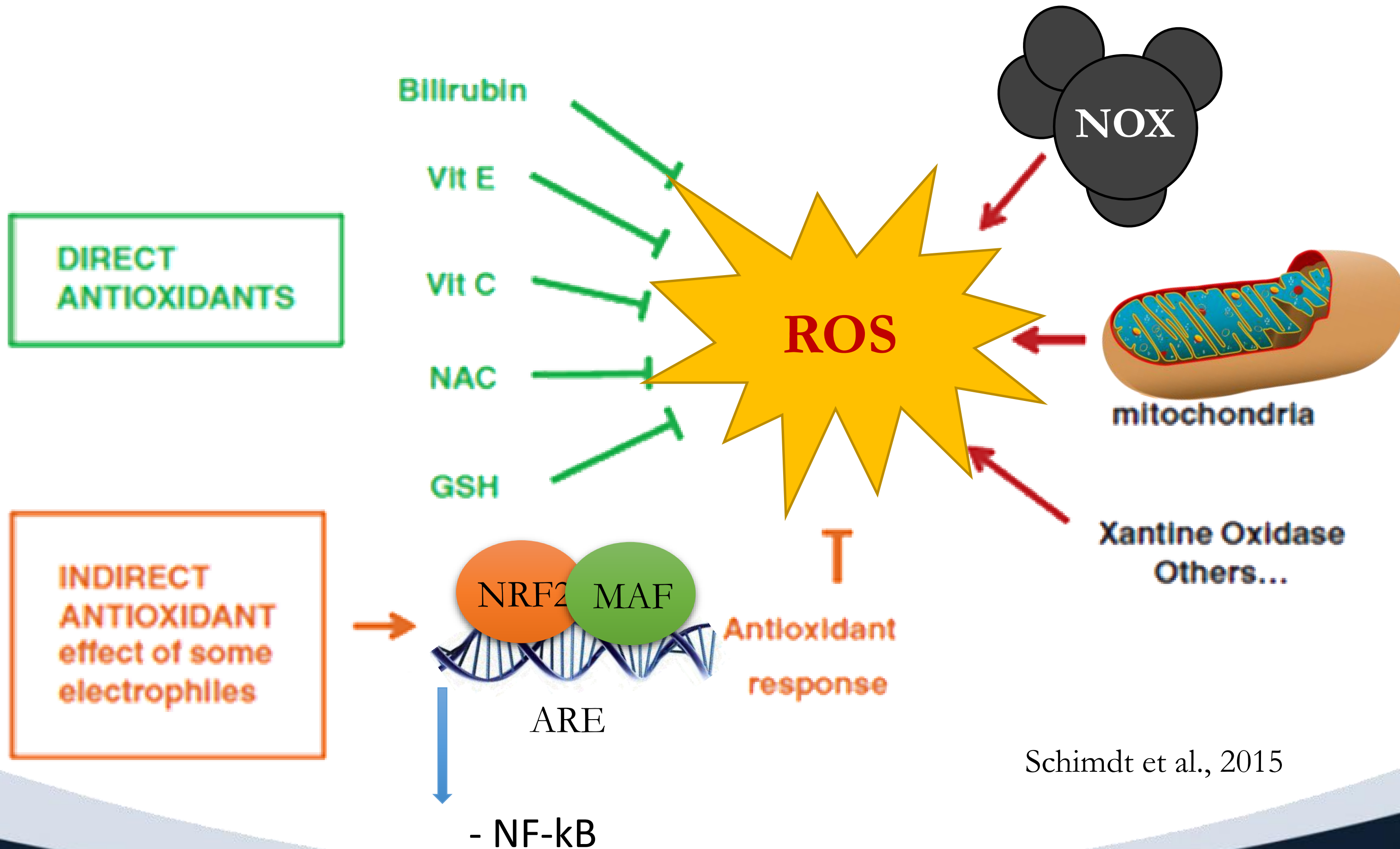
Classical direct mechanism



Indirect mechanism



DIRECT AND INDIRECT ANTIOXIDANTS



Schimdt et al., 2015

OXIDATIVE STRESS: CRITICAL POINTS

Feed

Lipid oxidation ANF
Mycotoxins

Animal

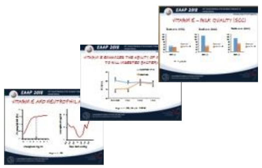
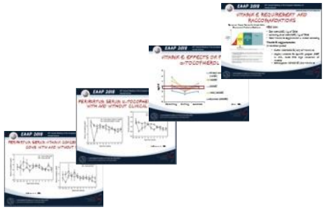
Physiological stage
>Transition period
Environmental stress (heat-transport)

Food

Antioxidant in milk
PUFA/CLA SOF
Rancidity

ANTIOXIDANT TIMELINE




1970s

- Prevent deficiency symptoms
- Prevent RFM
- Improve fertility

1980

- Antioxidants to counteract RFM and mastitis
- Controlled studies

1990

Immune response

- Neutrophil function
- PA activity

2000

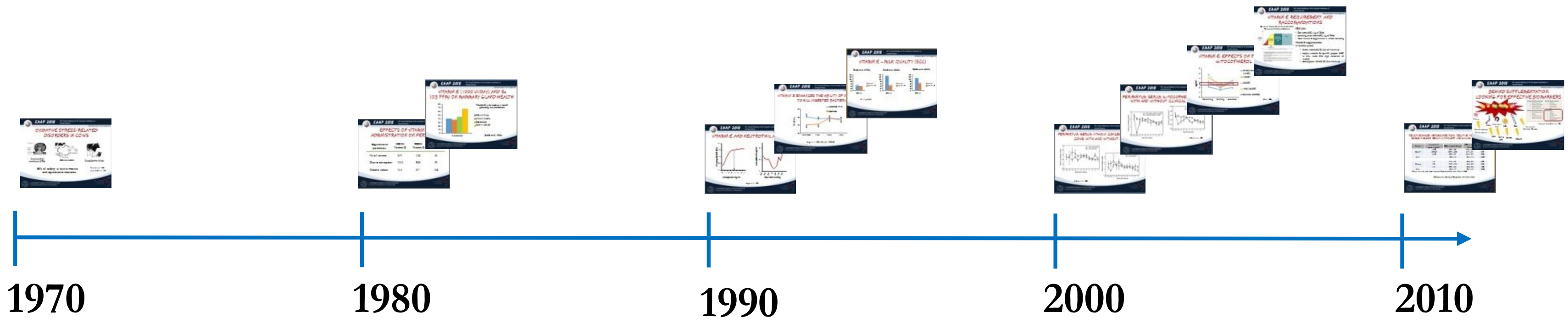
Epidemiological studies

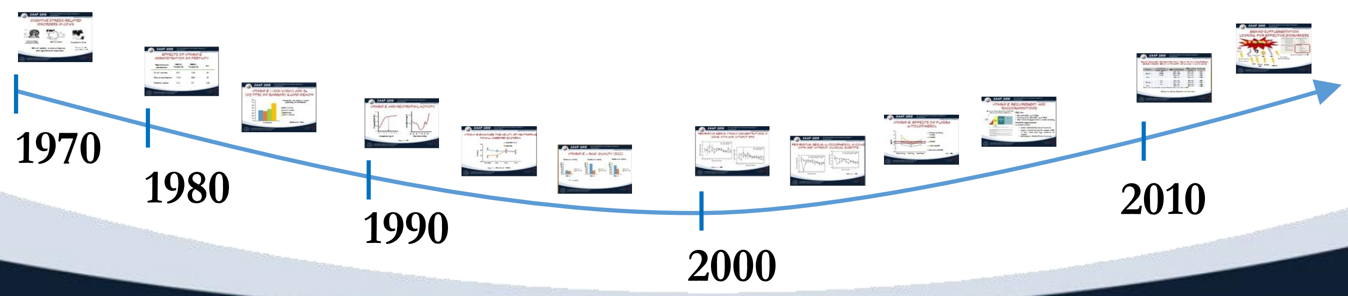
Re-visiting Vitamin E supplementation

2010

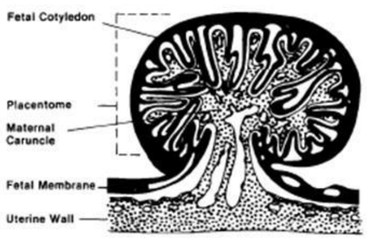
Biomarkers of antioxidant status

ANTIOXIDANT TIMELINE

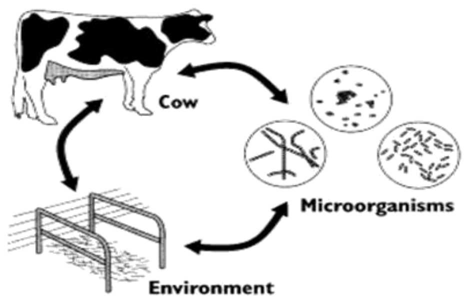




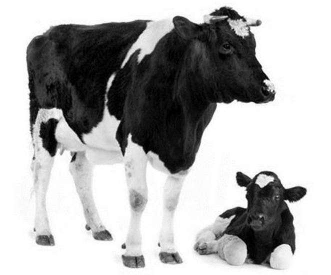
OXIDATIVE STRESS-RELATED DISORDERS IN COWS



Retained fetal membrane (RFM)



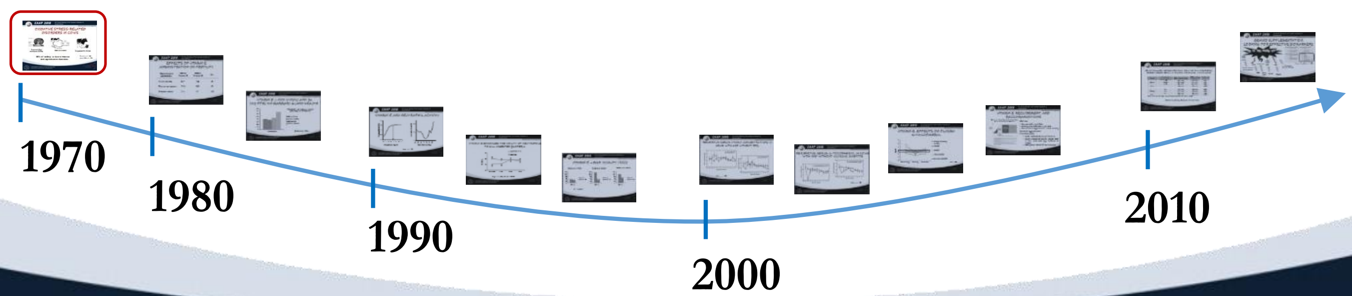
IMI and mastitis



Reproductive failure

Trinder et al., 1969
Julien WE et al., 1976

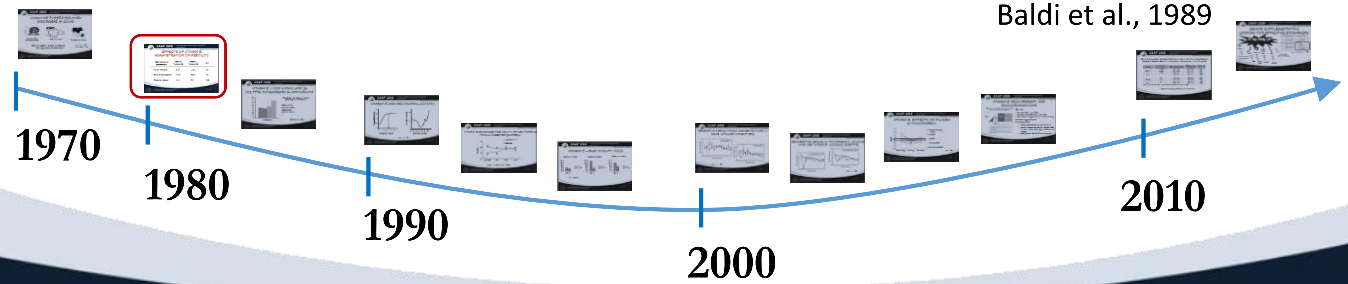
50% of culling is due to mastitis and reproductive disorders



EFFECTS OF VITAMIN E ADMINISTRATION ON FERTILITY

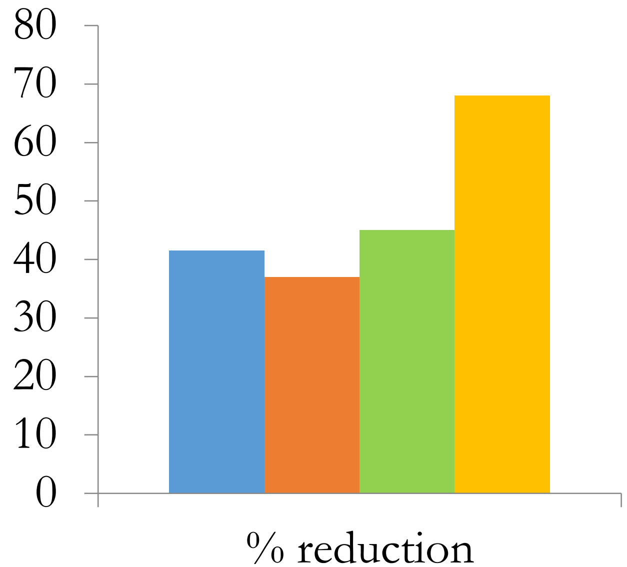
Reproductive parameters	1000 IU Vitamin E	2000IU Vitamin E	P<
Nr of services	2.17	1.32	.01
Days to conception	111.3	83.8	.01
Placenta release	11.4	9.7	NS

Baldi et al., 1989

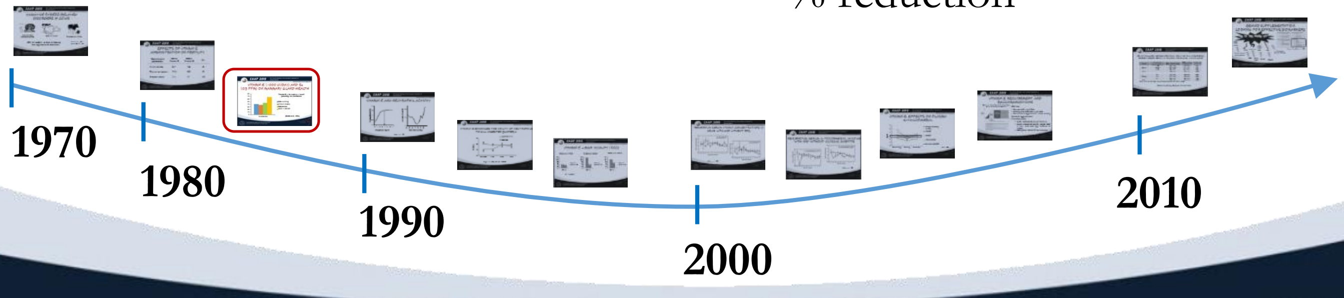


VITAMIN E (1000 UI/DAY) AND Se (0.3 PPM) ON MAMMARY GLAND HEALTH

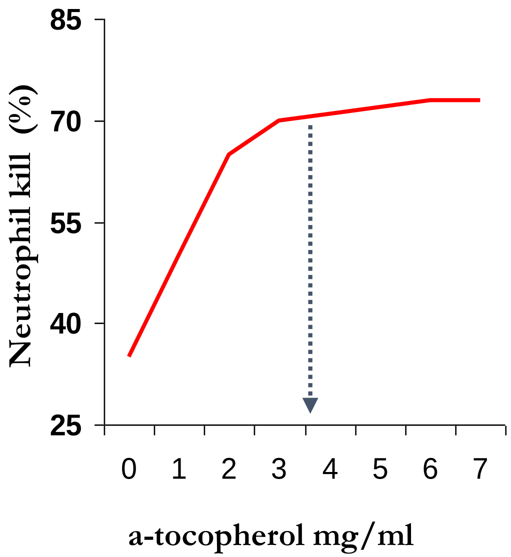
Vitamin E + Se work as a "team" protecting cell membranes

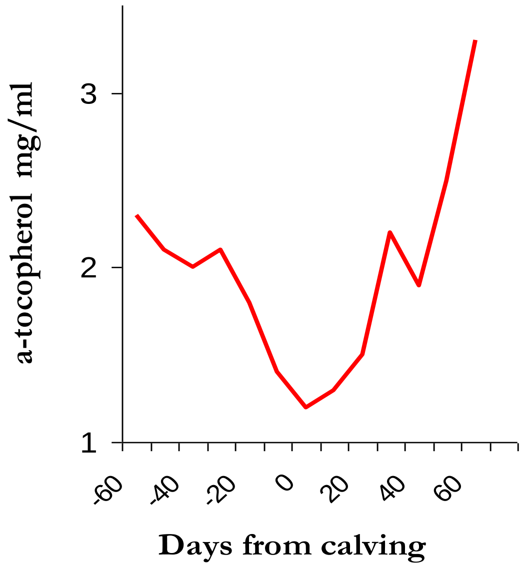


Smith et al., 1984

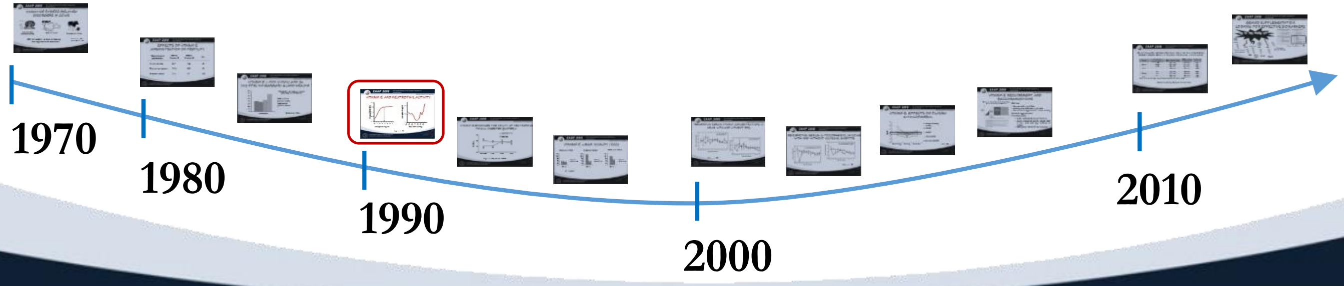


VITAMIN E AND NEUTROPHIL ACTIVITY

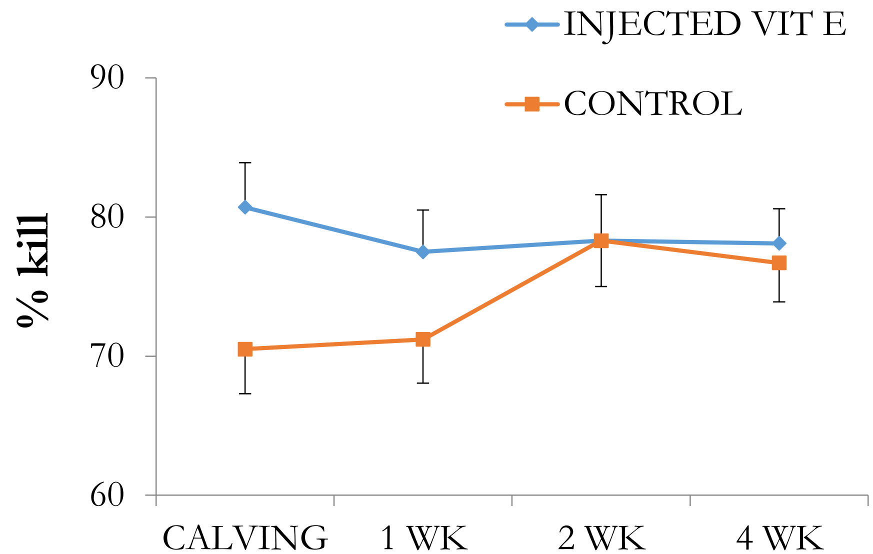




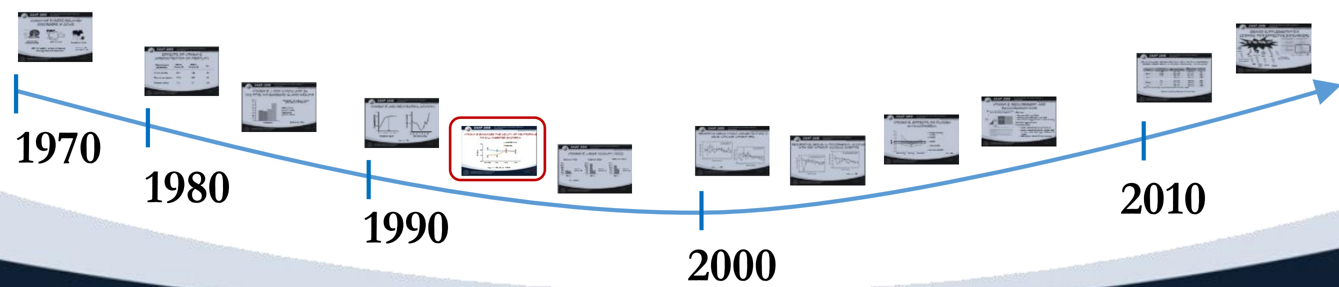
Hogan et al., 1993



VITAMIN E ENHANCES THE ABILITY OF NEUTROPHILS TO KILL INGESTED BACTERIA

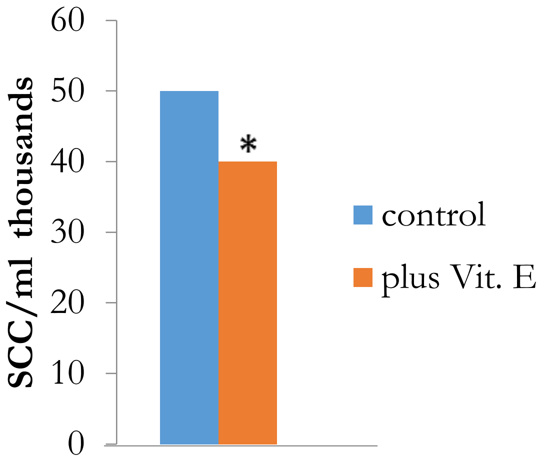


Hogan et al., 1992

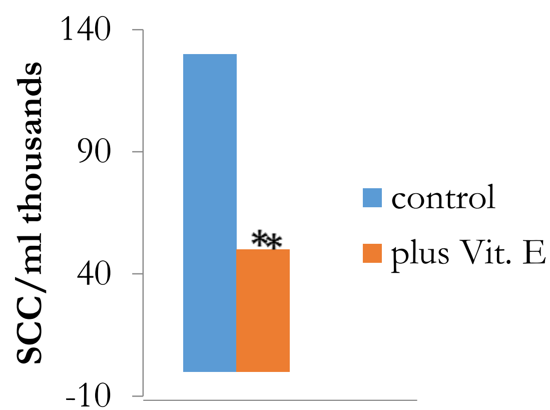


VITAMIN E – MILK QUALITY (SCC)

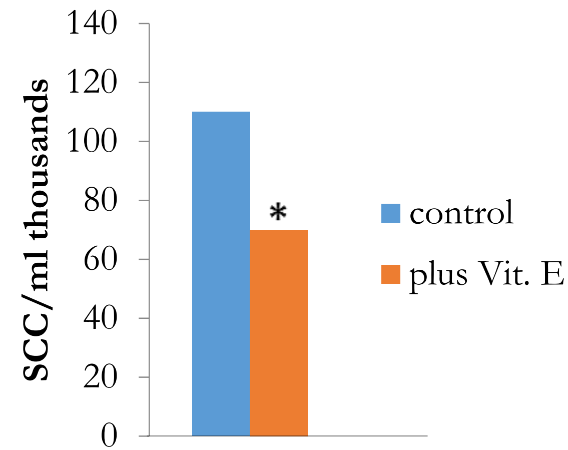
Batra et al. (1992)



Baldi et al. (2000)

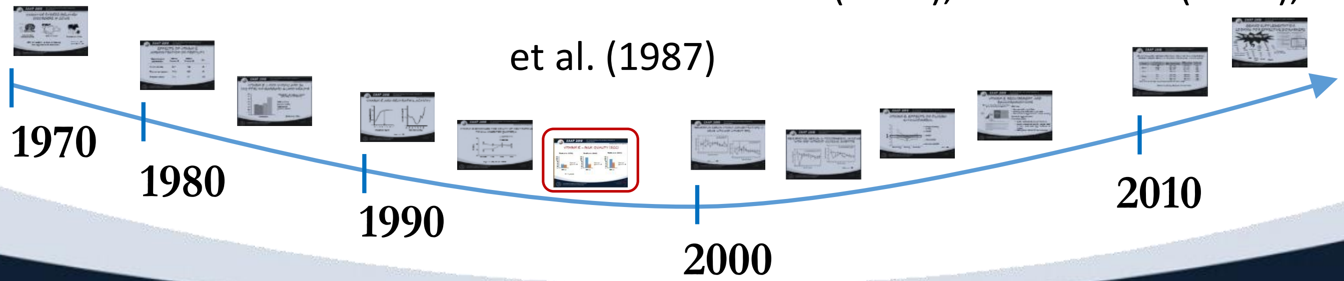


Politis et al. (2004)

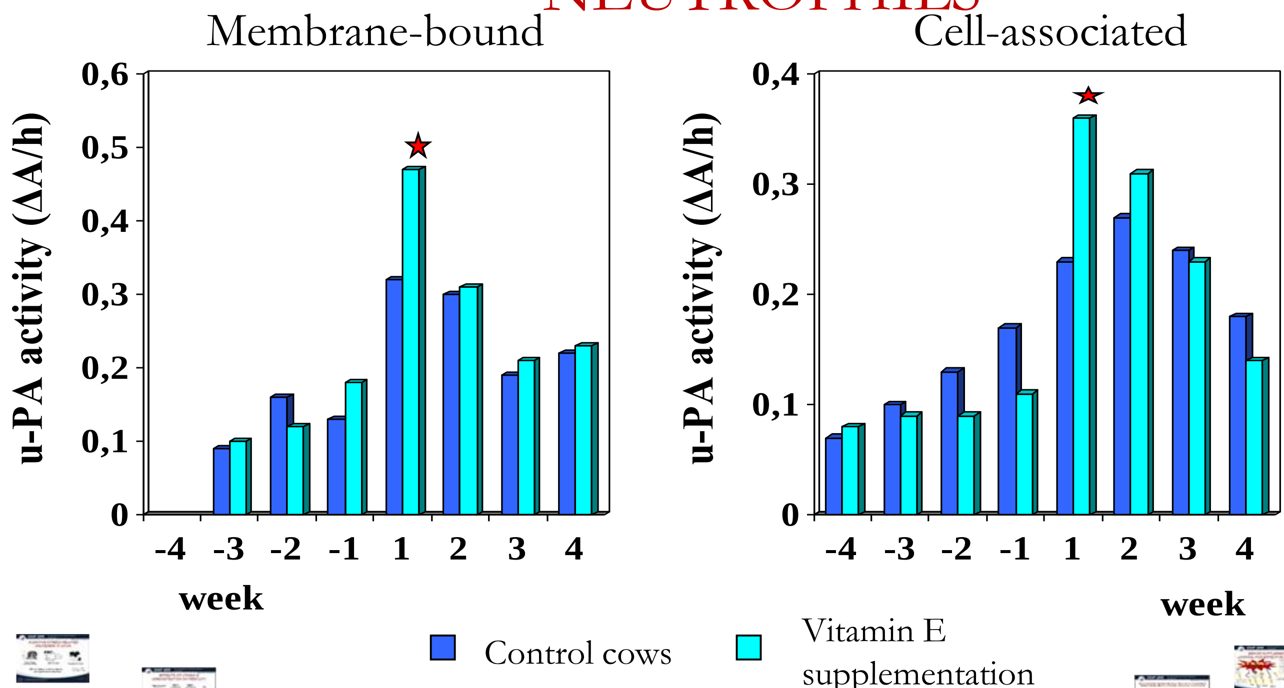


***p<0.05; **p<0.01**

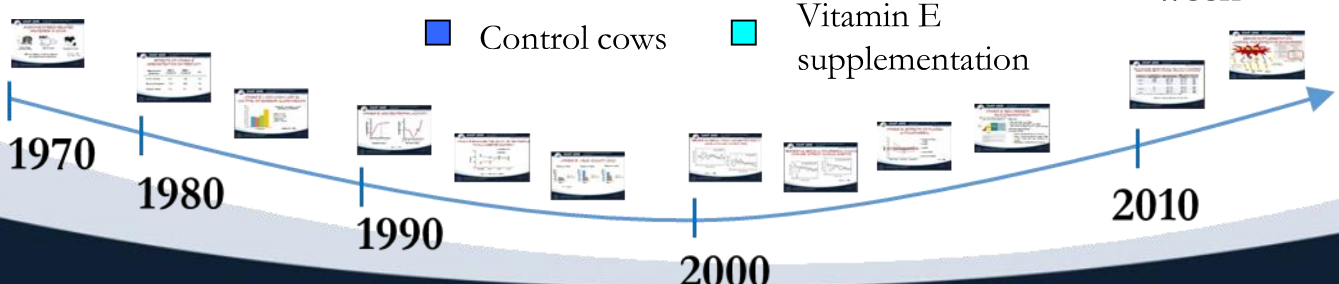
No effect Waller et al. (2007), Jukola et al. (1996), Erskine et al. (1987)



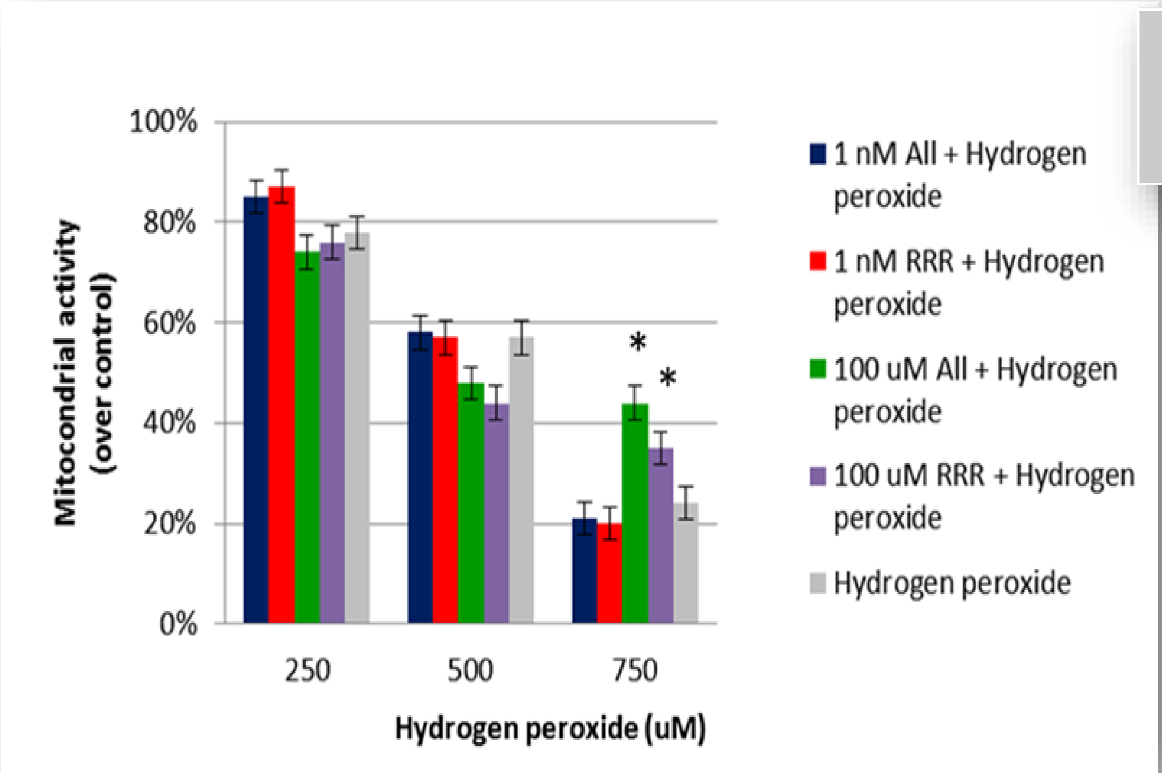
EFFECTS OF VITAMIN E ON Urokinase-PLASMINOGEN ACTIVATOR ACTIVITY IN PMA STIMULATED BOVINE NEUTROPHILS



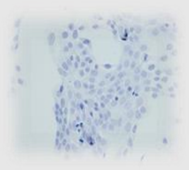
Politis et al., 2001



IN VITRO ANTIOXIDANT ACTIVITY



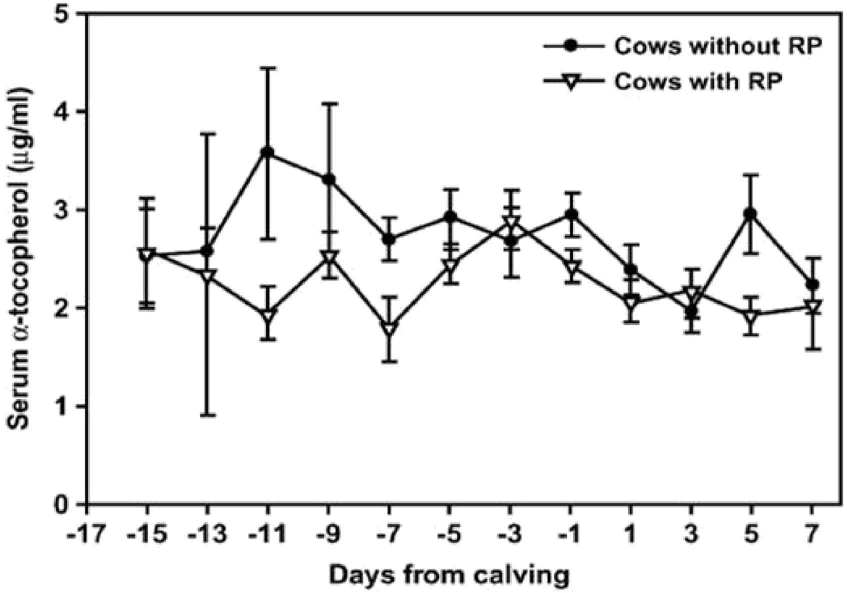
BME-UV1

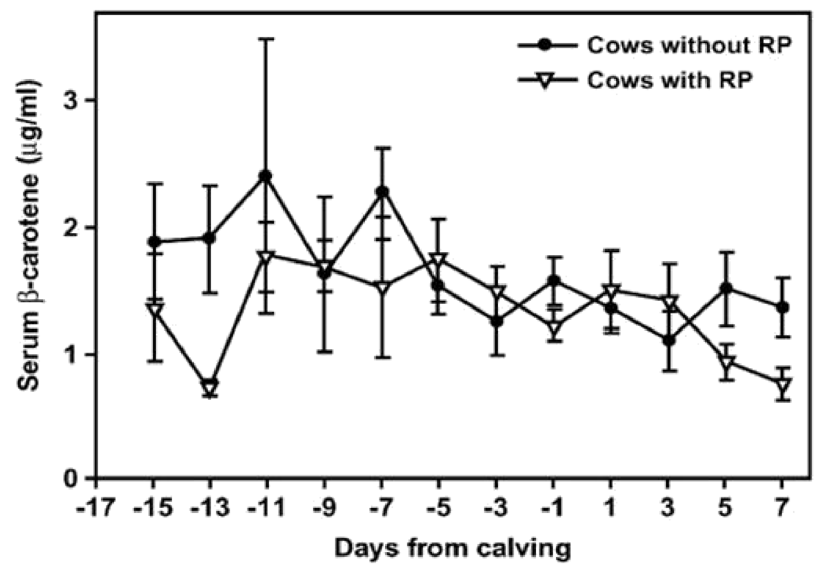


Mammary gland

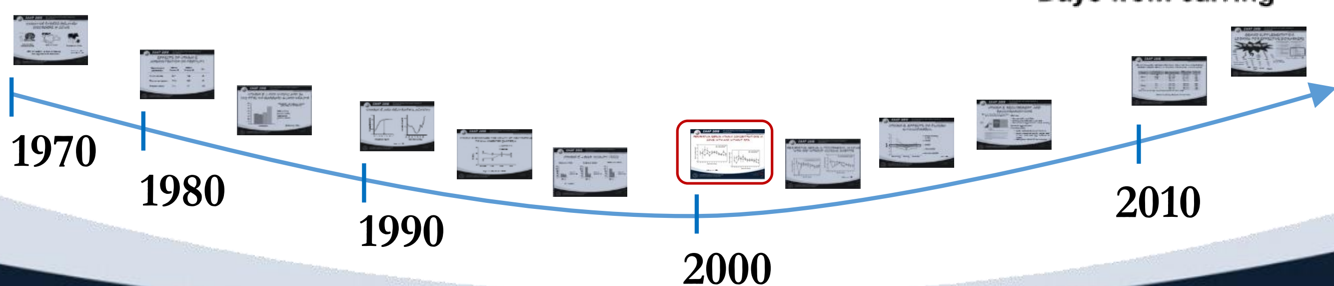
RRR α-tocopherol and **ALL-rac α-tocopherol** are able to counteract *in vitro* cytotoxic damage induced by oxidizing agents on bovine mammarty epithelial cells. (Baldi et al.2004)

PERIPARTUM SERUM VITAMIN CONCENTRATIONS IN COWS WITH AND WITHOUT RFM

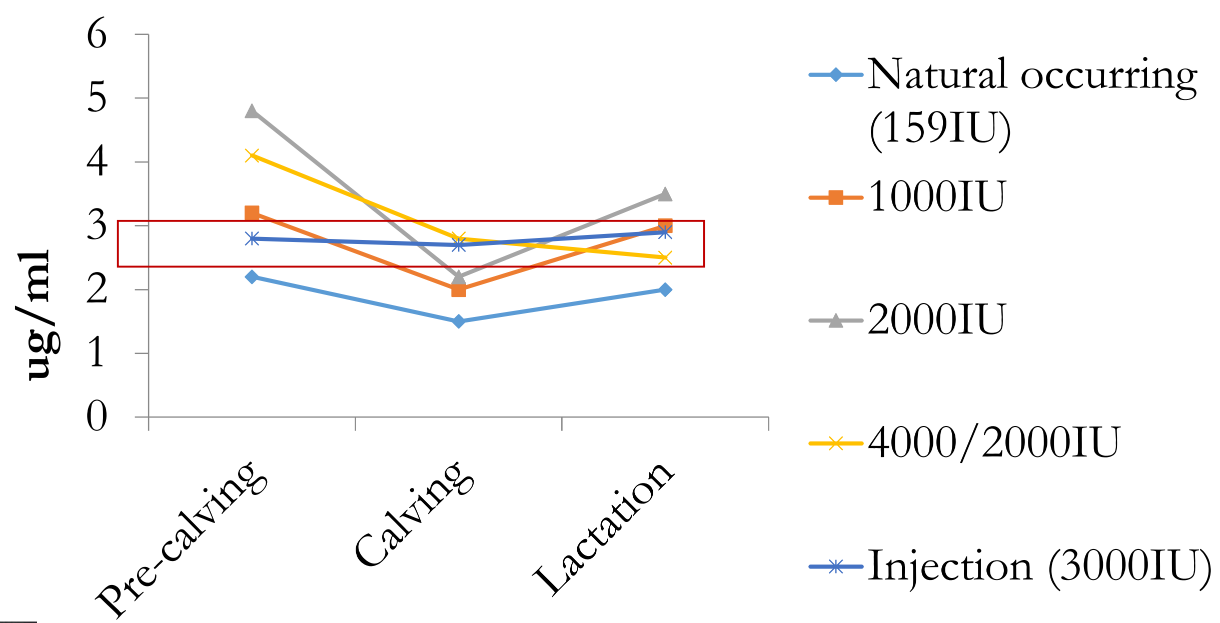




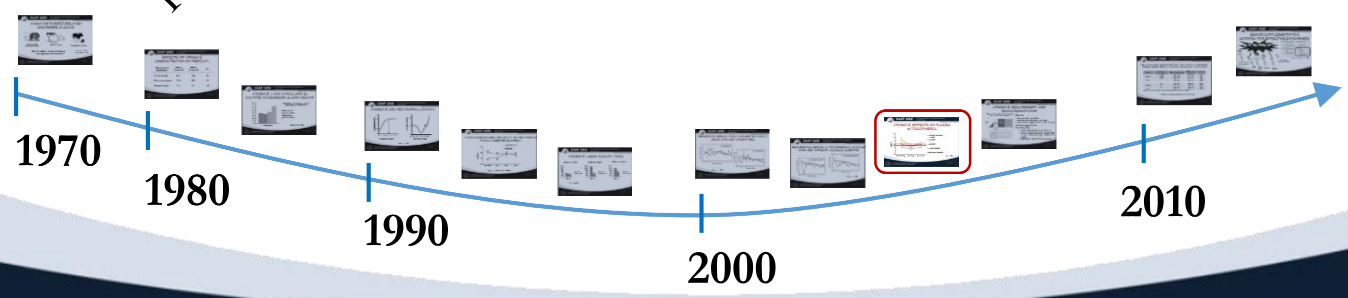
LeBlanc et al., 2004



VITAMIN E: EFFECTS ON PLASMA α-TOCOPHEROL



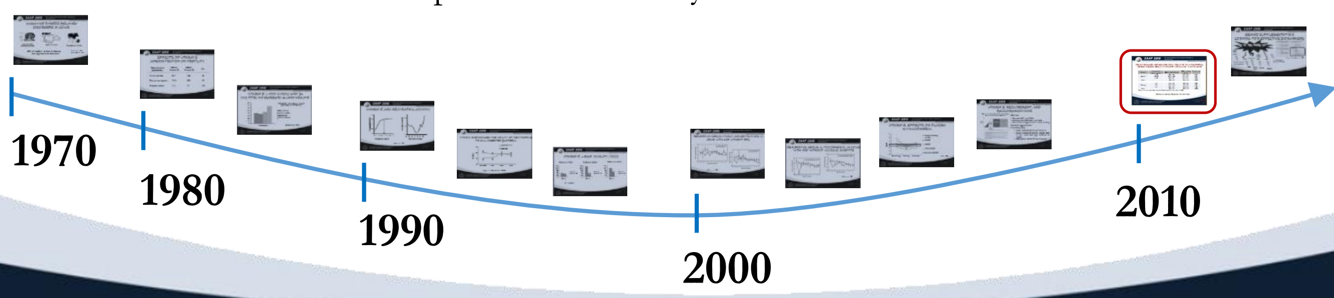
Baldi, 2005



REACTIVE OXYGEN METABOLITES (ROM) RELATIVE TO α-TOCOPHEROL LEVELS IN BLOOD SERUM AT DRY-OFF AND CALVING IN DAIRY COWS

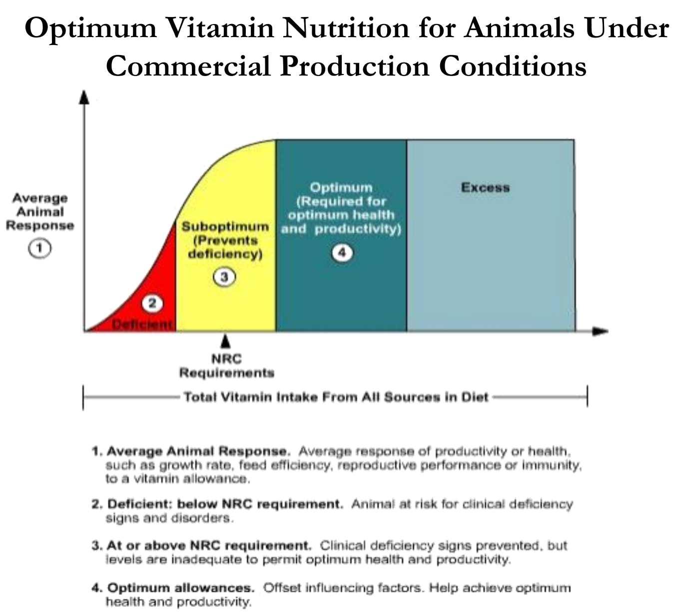
Period	α-tocopherol groups (μg/ml)	ROM at dry-off (U/ml)	ROM at calving (U/ml)	P within rows
Dry-off	> 6.25	$40.8^{a} \pm 3.2$	$49.6^{a} \pm 3.2$	0.754
	4.25-6.25	$53.3^{ab} \pm 2.09$	$61.1^{b} \pm 2.1$	0.137
	< 4.25	$56.2^{b} \pm 3.1$	$64.4^{b} \pm 3.1$	0.960
Mean		50.0 ± 1.6	58.4 ± 1.6	<0.001
Calving	> 3	$41.5^{a} \pm 2.9$	53.0 ± 2.9	0.078
	2-3	$51.5^{ab} \pm 2.5$	61.2 ± 2.5	0.106
	< 2	$58.3^{b} \pm 2.6$	62.0 ± 2.6	1.000
Mean		50.4 ± 1.5	58.7 ± 1.5	<0.001

[a,b]Means within the same column and period followed by different letters differ at $P < 0.05$



Politis et al., 2012

VITAMIN E REQUIREMENTS



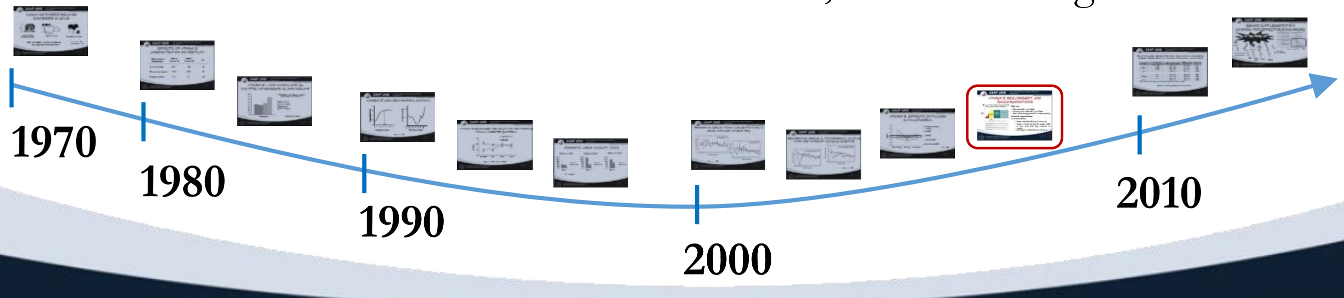
NRC 2001

- Dry cows 73IU/ kg of DMI
- Lactating dairy cows 18IU/ kg of DMI
- Total vitamin E supplemental + natural occurring

Vitamin E supplementation

(in transition period)

- Cows: 1000-2000 IU/day of vitamin E in dry period and 500-100 IU/day lactation
- Higher amounts for specific purpose (SOF in milk, herds with high incidence of mastitis)



BEHIOND SUPPLEMENTATION: LOOKING FOR EFFECTIVE BIOMARKERS

Protein Oxidation Products
- Protein Carbonyls
- Methionine Sulfoxide
- Tyrosine Products (nitro-, chloro-, o/m-, di-)

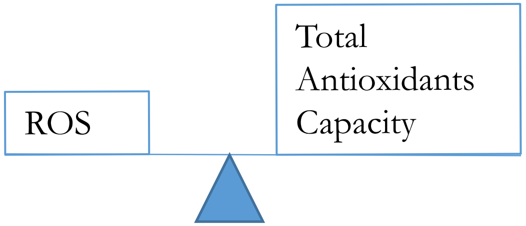
Lipid Peroxidation Products
- Lipid Hydroperoxide (LOOH)
- Thiobarbituric Acid Reactive Substances

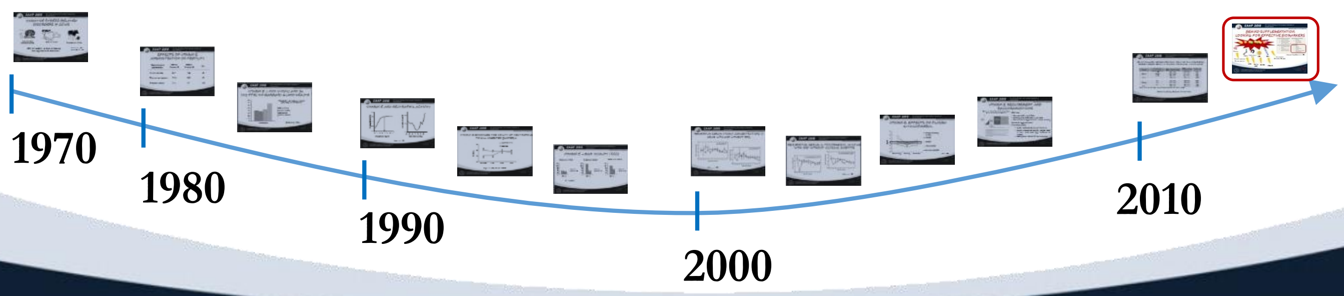
DNA Oxidation Products
- 8-OH-dG
- Strand Breaks (Comet Assay)
- M1G

Antioxidants
- Vitamin C
- Vitamin E
- Co-Enzyme $Q_{9/10}$
- Uric acid
- Total Antioxidant Capacity
- Cysteine/Cystine

Celi, 2011; Shah et al., 2014

Oxidative stress index (OSI) as an approach in ruminant and veterinary medicine to define protective nutritional strategies on the basis of antioxidant supplemention



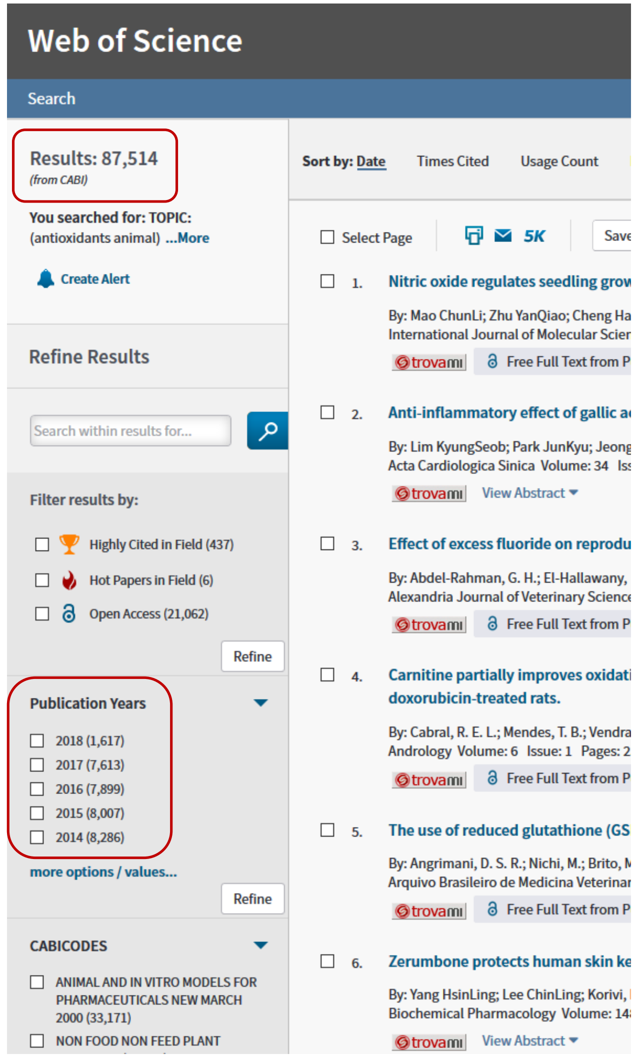


- Mantaining an active antioxidant network is recommended for improving the health and performance in several crucial phase.
- Nutrition can have a major influence on OS occurrence, since several antioxidant components are micronutrients and/or food-feed components
- Vitamin E & Se (but also Vitamind C, β-carotene, trace elements…) are known to be effective dietary antioxidants, while several others are under investigation and cannot yet be recommended as routine dietary supplements BUT could be a promising area of research
- It is necessary to provide useful OS markers able to give indication about the Ox-Aox status of the animals

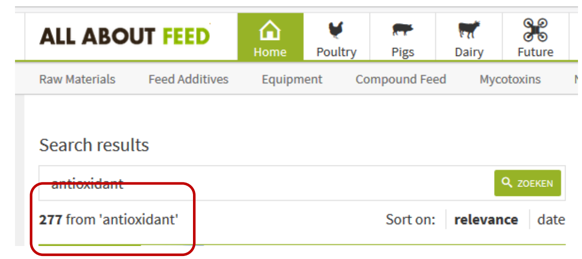
Baldi et al., 2007; Chauhan et al., 2014; Pinotti & Baldi 2015

Results >87000 articles

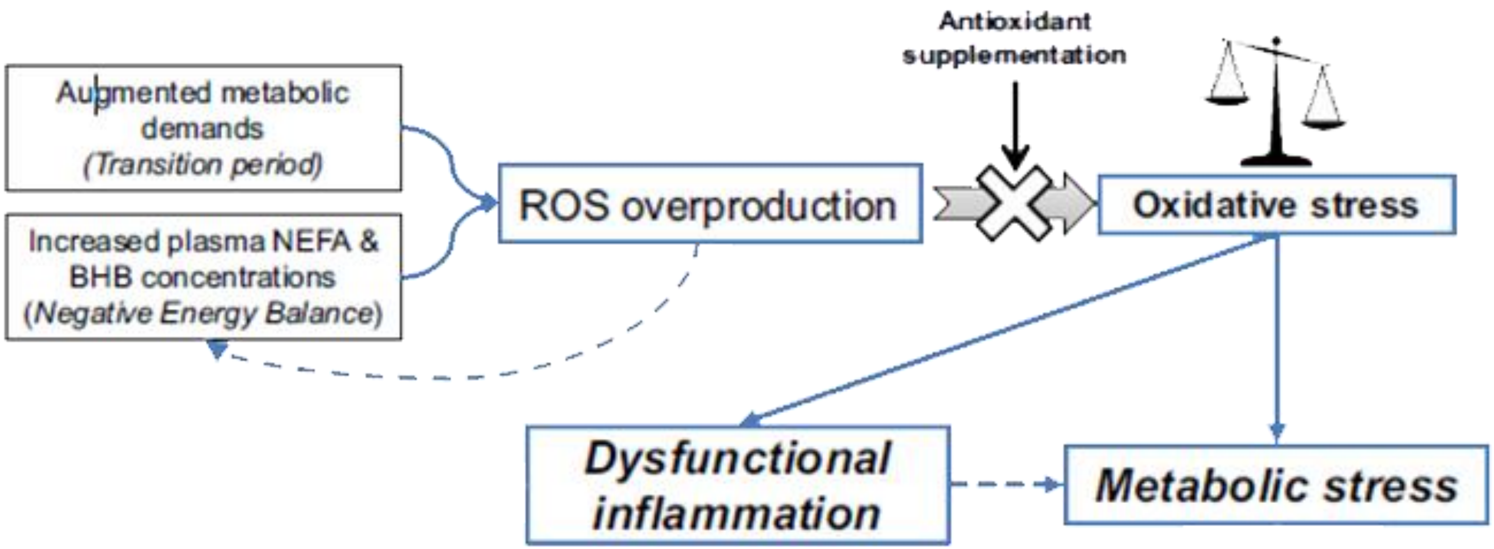
7000-8000 articles/year



Keywords: animal antioxidant



INTERPLAY BETWEEN ANTIOXIDANT SUPPLEMENTATION, METABOLIC STRESS, DYSFUNCTIONAL INFLAMMATION AND HEALTH DISORDERS

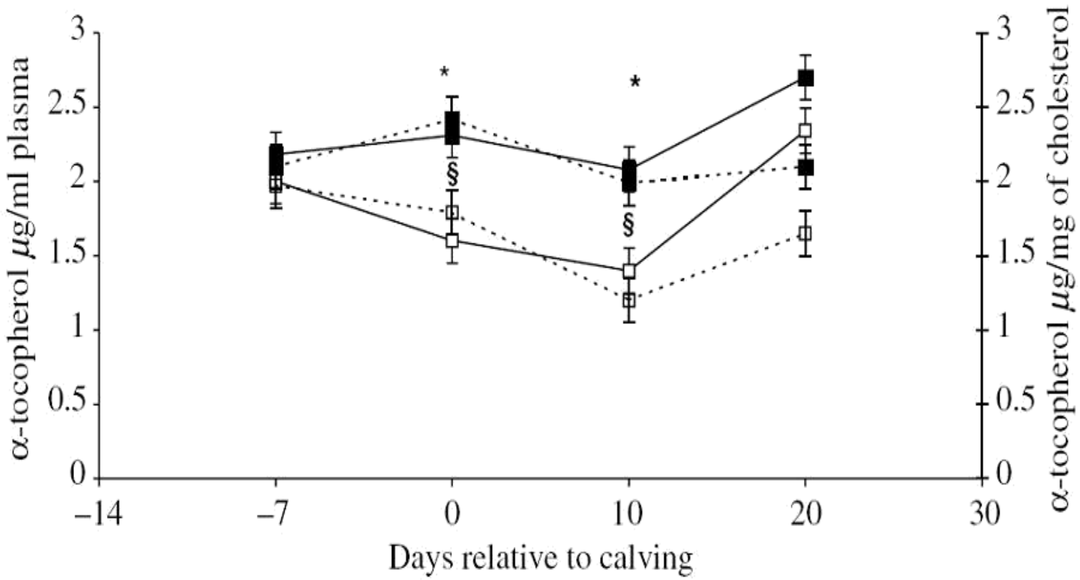


Abuelo et al., 2015

NEB and Oxidative Stress: the link between body fat mobilization and OS

- Cows with higher BCS loss after calving and greater plasma BHBA and NEFA had higher plasma ROMs and TBARS (Bernabucci, 2005).
- Cows in severe NEB during early lactation had increased OS, possibly due to the reduced availability of antioxidants (Perdenera et al., 2010).
- An oxidative challenge is not an issue when the antioxidant defense is in check and a mild inflammatory condition (especially in the liver) is a common situation (Bradford., 2015).
- May antioxidants help the liver to manage NEB and export NEFA to the mammary gland as VLDL?

RUMEN PROTECTED CHOLINE (20GR CHOLINE/D) SUPPLEMENTATION AND VITAMIN E STATUS IN TRANSITION DAIRY COW



□ no RPC+ 1000 IU vitamin E

■ 20g RPC +1000 IU vitamin E

Pinotti, 2003;
Baldi, 2005

VITAMIN E – LIVER FUNCTION AND METABOLIC DISEASES

- Link between Vitamin E/antioxidant status and metabolic status in the liver.
- Cows with fatty liver have lower AOX status than healthy ones (Mudron, 1999) and lower plasma Vitamin E.
- Rumen protected choline adimistered to dairy cows induced lower NEFA and higher Vitmain E that could be related to a better liver function and lipid hepatic metabolism

ASSOCIATIONS BETWEEN BLOOD FATTY ACIDS, β-HYDROXYBUTYRATE, AND α-TOCOPHEROL IN THE PERIPARTURIENT PERIOD IN DAIRY COWS: AN OBSEVATION STUDY

- A total of 131 Holstein cows from 4 commercial farms
- Plasma samples analyzed for α-tocopherol, NEFA, BHB and total cholesterol in serum samples
- Measurement of ROS and SAC in serum samples; calculation of the Oxidative Stress Index (OSi) ROS/SAC

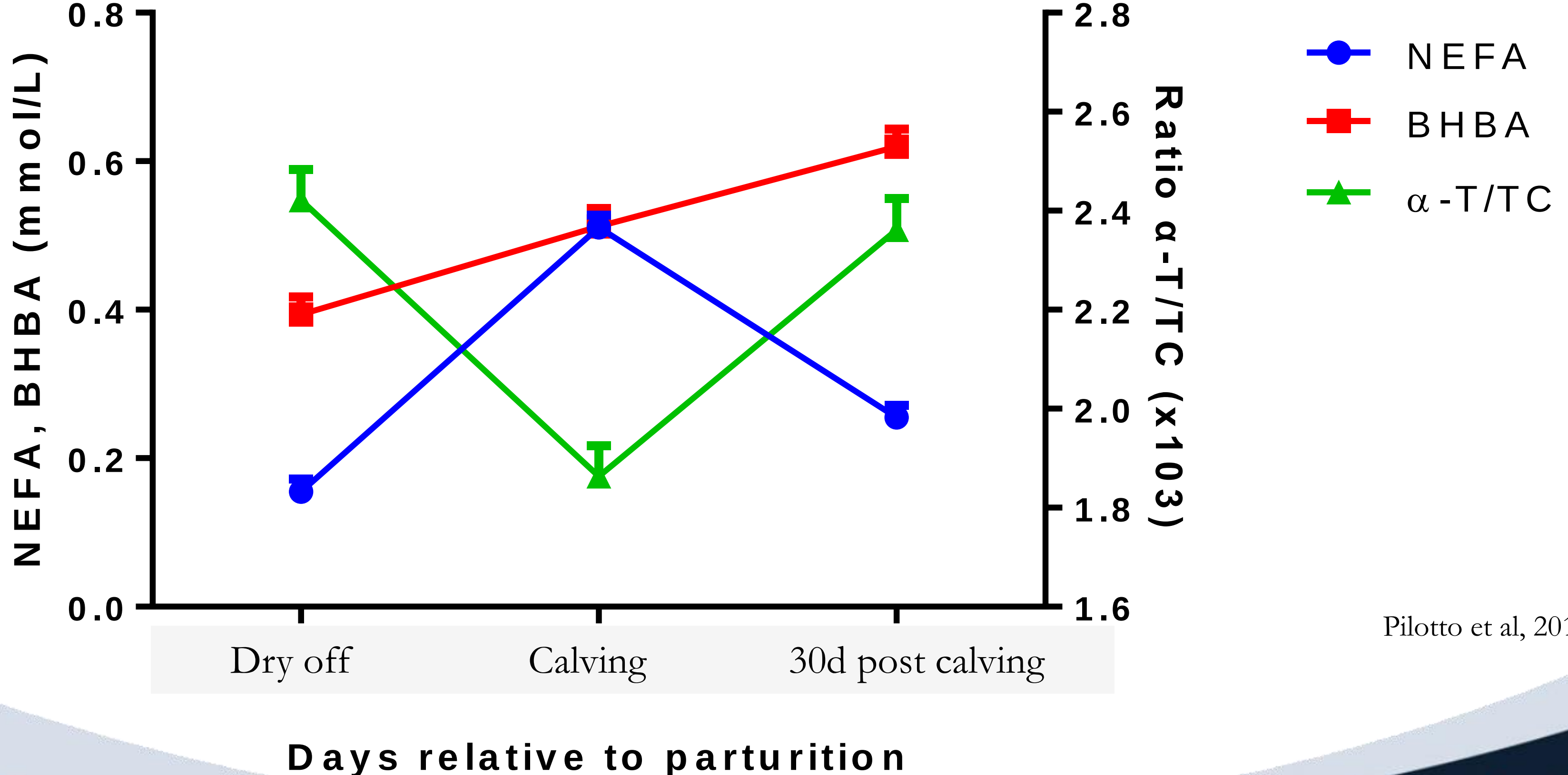
Pilotto et al., 2016

CHANGES IN LEVELS OF BLOOD FFA, BHB, α-TOCOPHEROL (αT) LEVELS AND THE RATIO OF αT AND TOTAL CHOLESTEROL (TC) IN BLOOD SERUM AT DRY-OFF, CALVING AND 30D POSTPARTUM

Period	FFA (mmol/L)	BHB (mmol/L)	αT (mmol/L)	αT/TC
Dry-off	0.155[a]+/- 0.01	0.39[a] ± 0.02	3.89[a] ± 0.09	2.42[a] ±0.06
Calving	0.511[b] ± 2.09	0.51[b] ± 0.02	2.47[b] ± 0.09	1.86[b] ±0.06
30d postpartum	0.255[c]+/- 0.02	0.62[c] ± 0.03	3.98[b] ± 0.1	2.36[a] ±0.06

Pilotto et al., 2016

NEFA – BHB DURING TRANSITION PERIOD



Pilotto et al, 2016

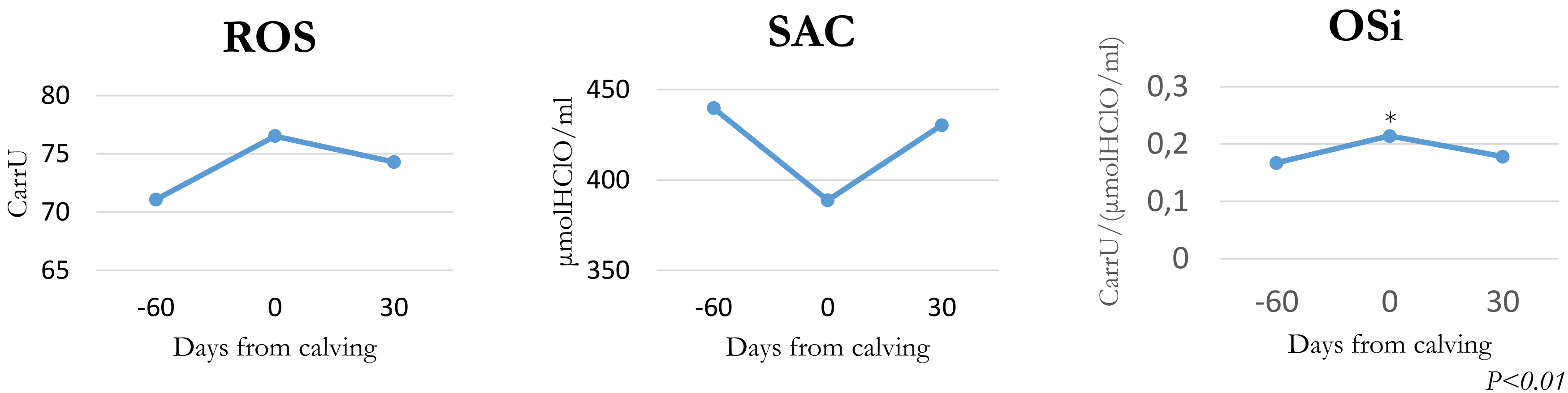
CORRELATIONS BETWEEN NEFA, BHBA, A-T AND THE RATIO OF A-T TO TOTAL CHOLESTEROL (TC) DURING THE PERIPARTURIENT PERIOD

			NEFA	BHBA	α-T	α-T/TC
Dry off	**NEFA**	Rho	1	0.114	-0.169	-0.002
		P	-	ns	ns (P=0.057)	ns
	BHBA	Rho		1	-0.370	-0.352
		P		-	***	***
	α-T	Rho			1	0.348
		P			-	***
	α-T/TC	Rho				1
		P				-
30 d postpartum	**NEFA**	Rho	1	-0.030	-0.300	0.028
		P	-	ns	***	ns
	BHBA	Rho		1	-0.104	-0.188
		P		-	ns	*
	α-T	Rho			1	0.388
		P			-	***
	α-T/TC	Rho				1
		P				-

* Correlation is significant at P <0.05 (2-tailed).
*** Correlation is significant at P< 0.001 (2-tailed).
ns: not significant

Pilotto et al., 2016

MEASUREMENT OF ROS AND SAC IN SERUM SAMPLES; CALCULATION OF THE OXIDATIVE STRESS INDEX (OSI)



- OSi was able to detect differences between different time points in transition period

Concentrations of α-tocopherol were lowest at calving, no differences in α-tocopherol concentrations at dry-off or 30d postpartum.

Negative correlations between fatty acids and α-tocopherol.

Negative correlations (stronger at dry-off and weak at 30d postpartum) between BHB and α-tocopherol after adjustment with cholesterol.

Overall these data show a significant correlation between NEB biomarkers and Vitamin E status.

TAKE HOME MESSAGE

- The transition period imposes quick metabolic changes that include the ones related to AOX system
- The challenge is to mantain red-ox homeostasis, not only pushing up the antioxidant system but also reducing ROS load, combining different nutritional, enviromental and mangement practice with a multidisciplinary approach.
- One strategy is to use of synergic nutrients (Vitamin E, Choline, Met) monitoring aox capacity of the animls/herd.
- Do not forget the offsprings!

Acknowledgments

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

Ioannis Politis
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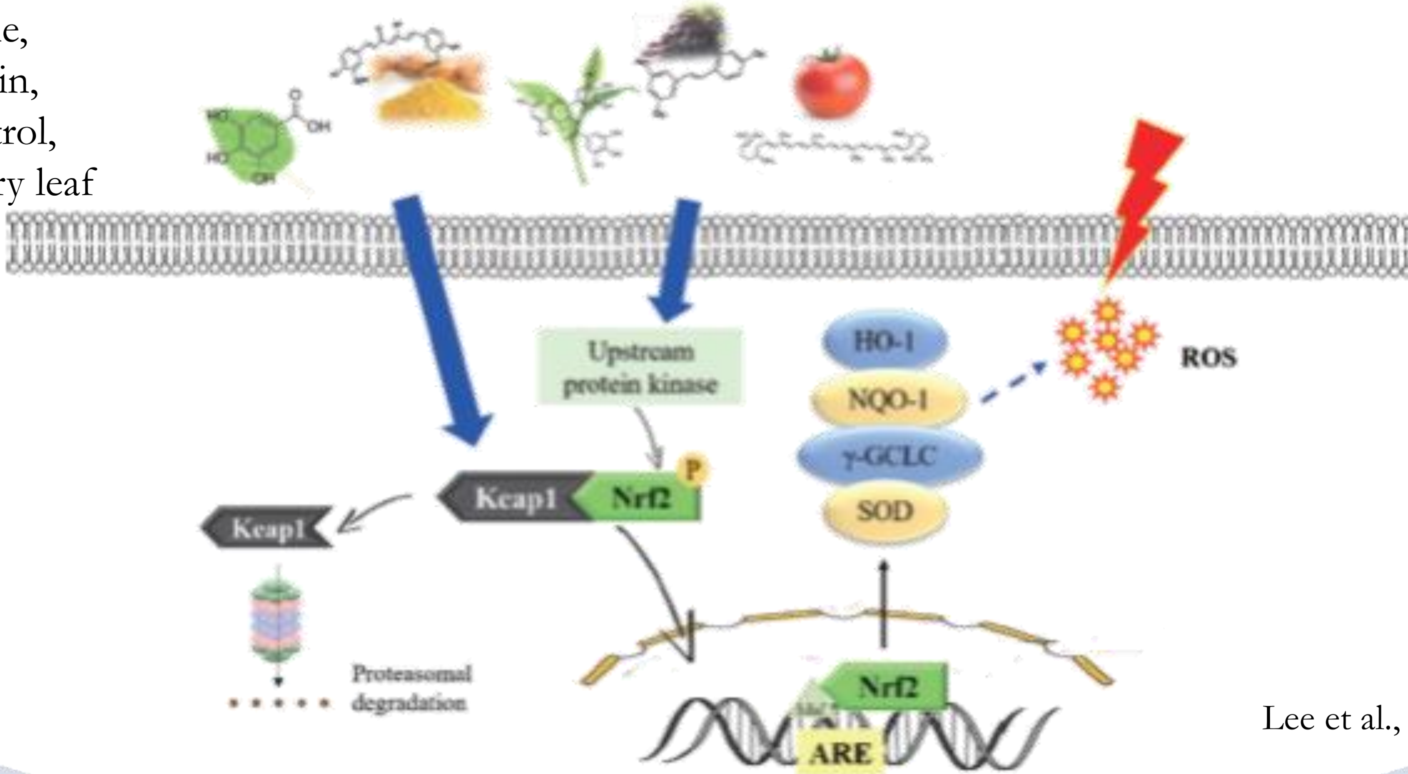
"ALTERNATIVE" ANTIOXIDANTS

Families	Sources	Antioxidant System	Other benefits
Carotenoids (Lutein, Lycopene)	Corn, fruit, tomatoes	Singlet oxygen quencer, secondary stabilizer other antioxidants	Immune system response
Plant secondary metabolites -Flavonoids (Flavonols, Flavonones) -Phenols -Sulfides/Thiols	Grapes, apples, citrus food, grains Apples, citrus fruits Garlic, onions, rosmarin, thymol [essential oils]	Chain breaker, metal chelator, scavenger free radicals and superoxide. Influences on enzymatic reactions Chain breaker and metal chelator -	Hepato-protector Immune system response Influences on rumen microbial fermentations

MOLECULAR MECHANISM OF PHYTOCHEMICALS

- catechin,
- lycopene,
- curcumin,
- resveratrol,
- mulberry leaf

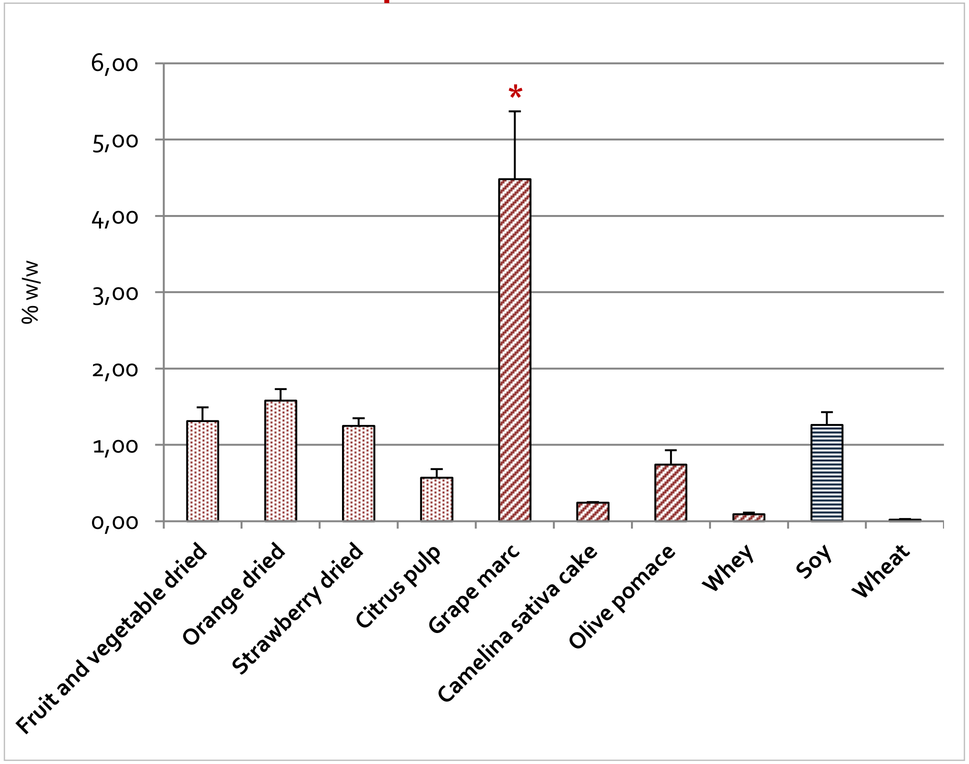


Lee et al., 2017

AFW and by-products could be considered a **promising bioaccessible source of antioxidants and phenolic** compounds

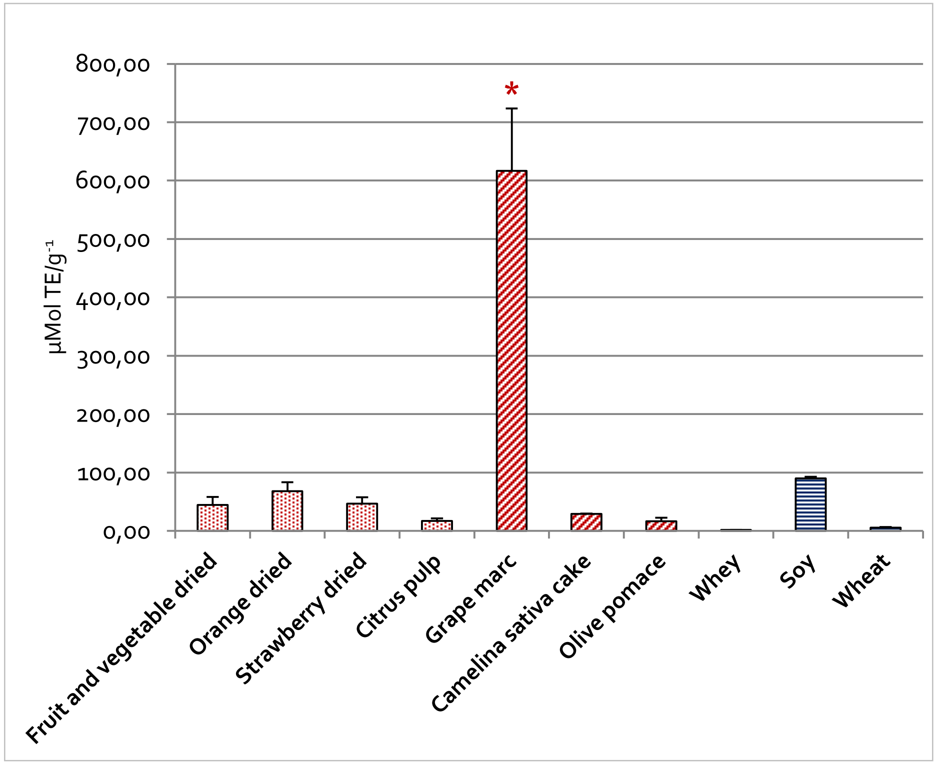
Agri-food waste - by-products	N. sample
Fruit and vegetables waste (FVW)	3
Orange dried	3
Strawberry dried	3
Citrus pulp	3
Grape marc	3
Camelina sativa cake	3
Olive pomace	3
Whey	3

Total phenolic content



* p>0.05

Antioxidant activity



* p>0.05

Positive Correlation ($r=0.95$; $p<0.001$) between the total phenolic content and antioxidant activity

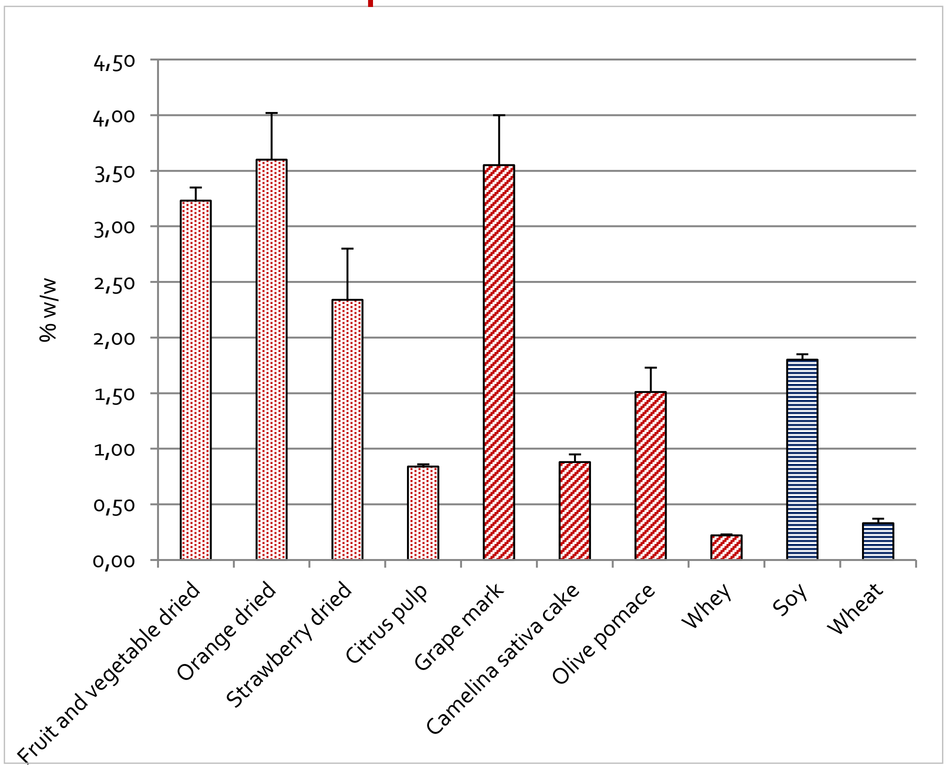
Positive Correlation ($r=0.75$; $p<0.05$) between the total phenolic content with chemical extraction and total phenolic content with in vitro extraction

Results and Discussion - Physiological Extraction and IVD

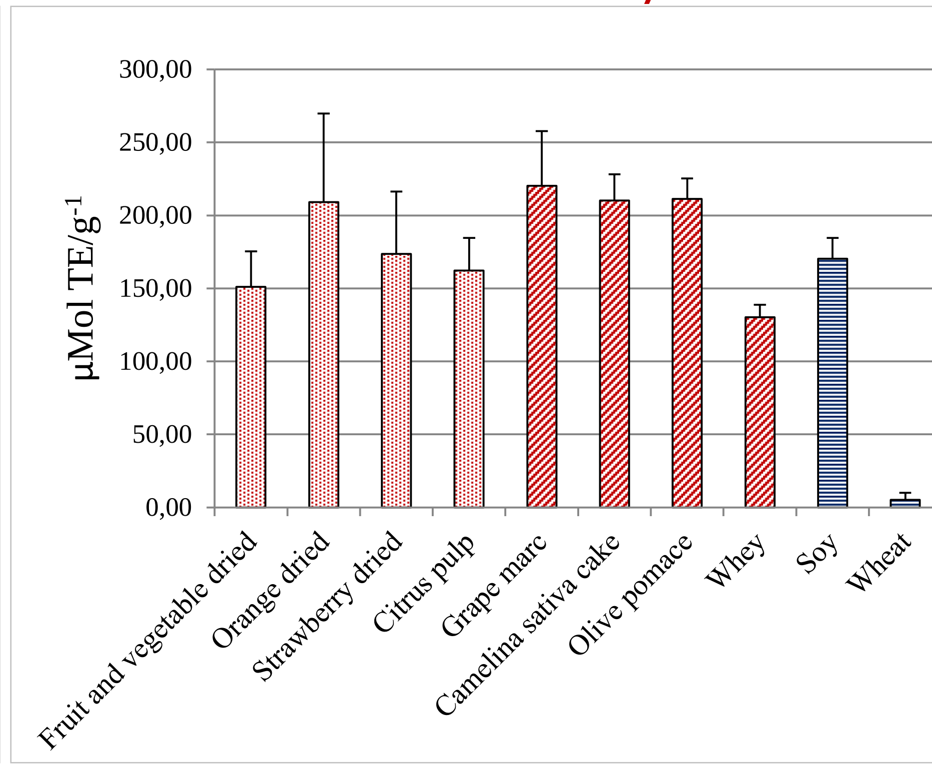
IVD

FVW	Orange dried	Strawberry dried	Citrus pulp	Grape marc	Camelina sativa cake	Olive pomace	Whey	Soy	Wheat
80% DM	90% DM	88% DM	82% DM	44% DM	66% DM	N.D.	98% DM	77% DM	75% DM

Total phenolic content

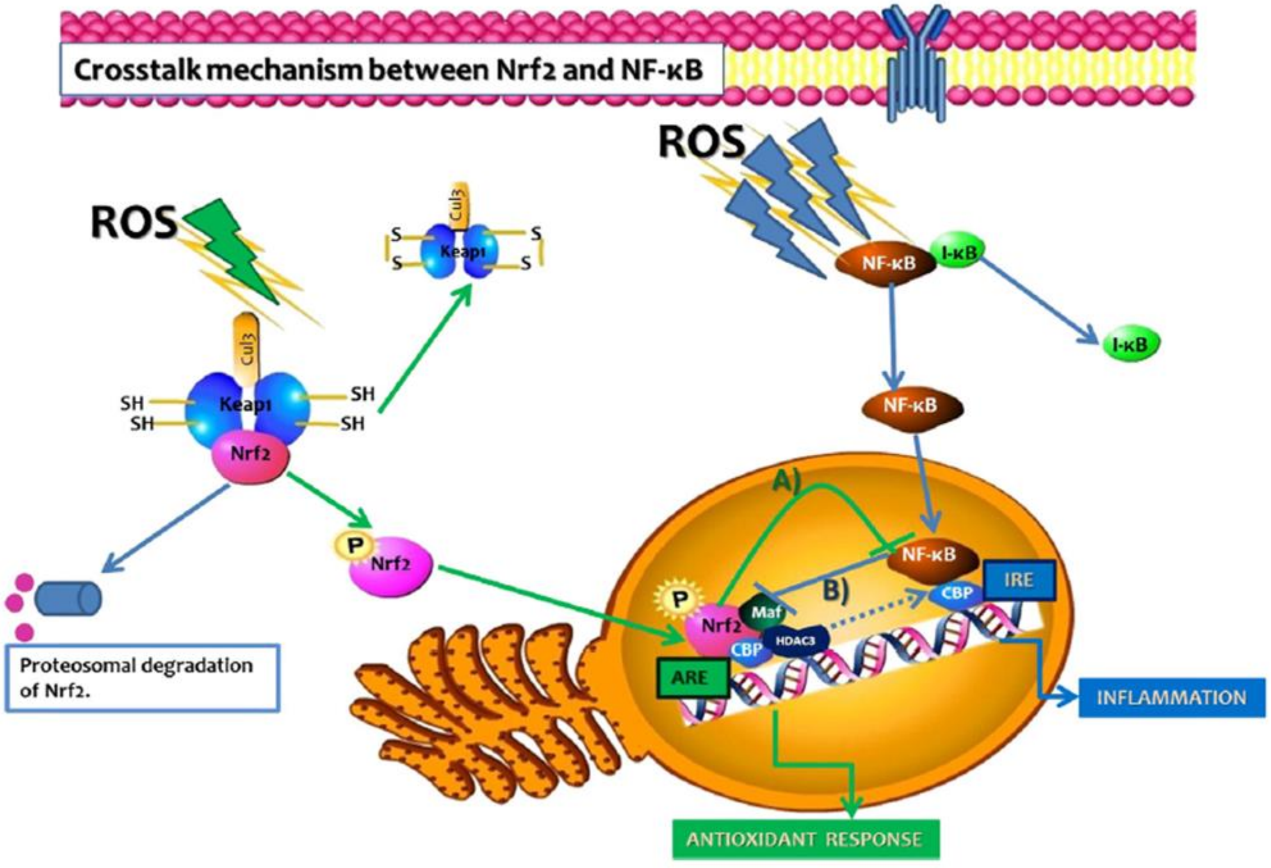


Antioxidant activity



The IVD showed higher total phenolic content and antioxidant capacity, suggesting that during the digestion the bioaccessibility of phenolic and antioxidant compounds was improved

EVIDENCE OF CROSS TALK BETWEEN Nrf2 and NF-kB



Adapted from Buelna-Chontal and Zazueta, 2013

THANK YOU

PRODUCTION: HIGH-PRODUCING DAIRY COWS AND OS

Group	No.	Milk yield (Kg/d)	Milk energy (MJ/d)	Lipohydroperoxide (μM)
I	5	34.2	122.2	3.6
II	6	51.7	164.8	6.5
P		0.001	0.021	0.015

High milk yield can be associated with OS indicated by oxidative modifications of circulating lipids

Marked increase in superoxide formation

(Lohrke et al., 2005)

EFFECTS OF INCLUSION OF SELENIUM-ENRICHED YEAST IN THE DIET OF LAYING HENS ON PERFORMANCE, EGGSHELL QUALITY, AND SELENIUM TISSUE DEPOSITION (Inverniizzi et al.,2013)