How to prevent an exaggerated inflammatory response in the transition period of dairy cows

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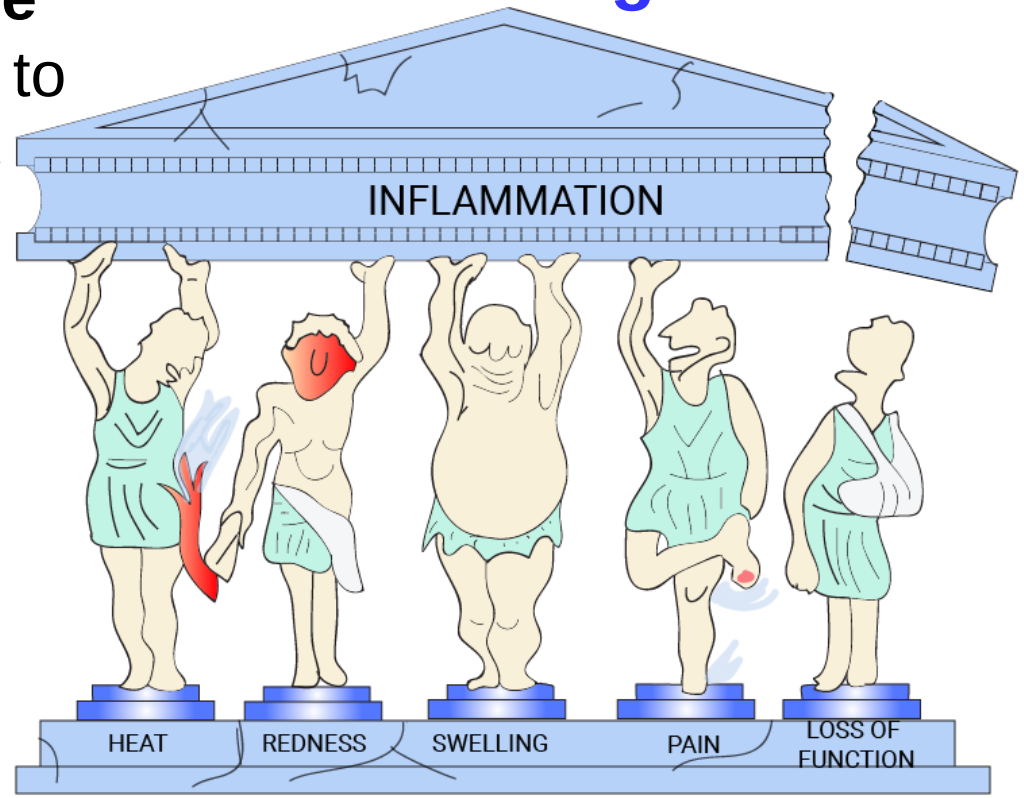
Summary

1. **inflammation: a frequent event of life**
   - **Local and systemic effects**
   - **Implication at the Liver level**
2. **inflammation: when could be defined exaggerated?**
   - **Acute and chronic indexes**
   - **Assessment of inflammation severity in TP**
   - **Causes in TP**
3. **Strategies to counteract inflammation at TP**
4. **Implications for the future**

Inflammation

- ✓ **Local protective response** of living mammalian tissue to injury due to **any etiologic agent (biotic & abiotic)**
- ✓ Aim of body defense reaction: to **eliminate or limit the spread** of **injurious agents**
- ✓ 2 basic processes with some overlapping:
  - **early inflammatory response**
  - **healing**

Cardinal signs



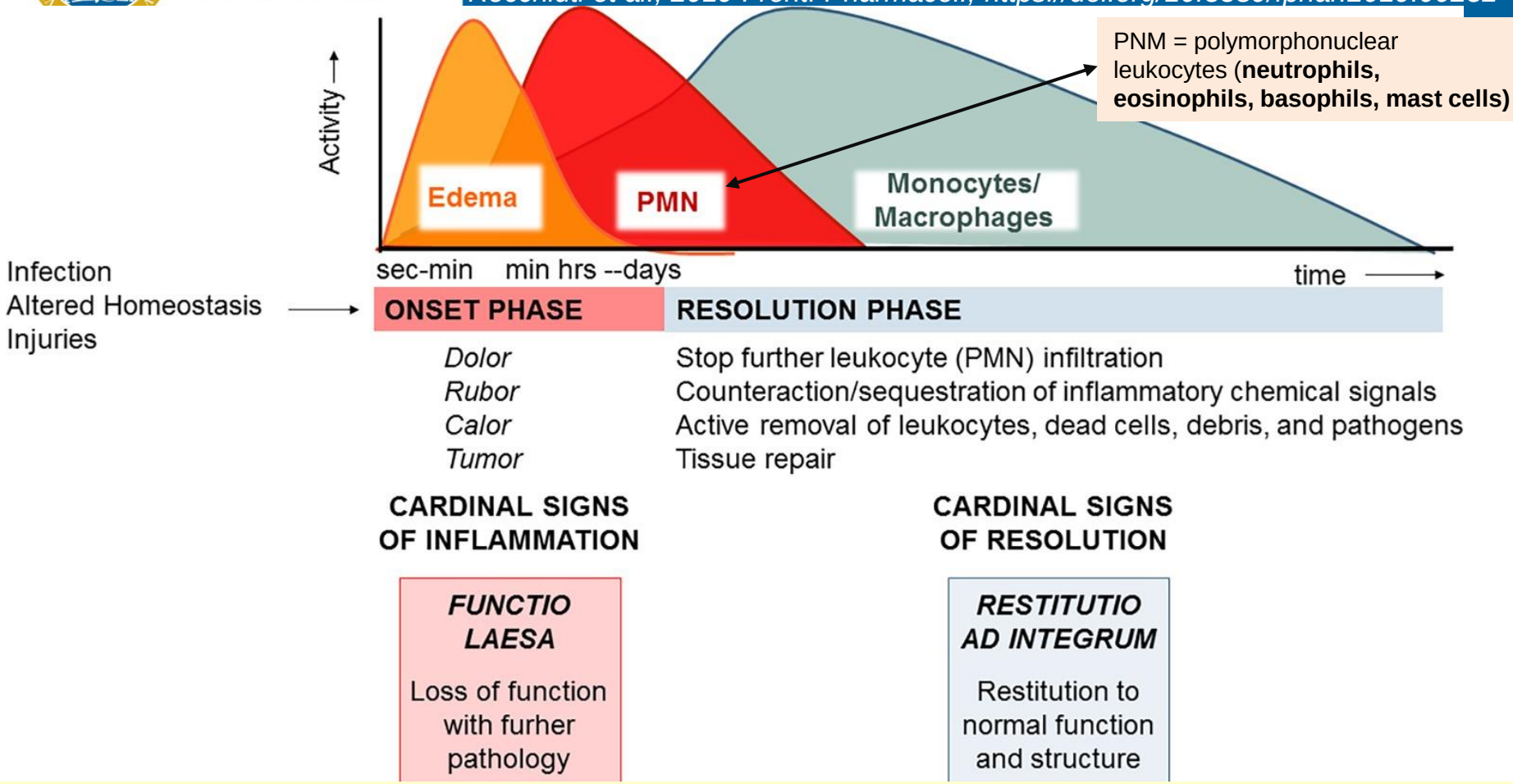
CALOR	RUBOR	TUMOR	DOLOR	FUNCTIO LAESA
Local hypothermia, fever	Hyperemia	Tissue swelling	Burning pain	

CELSUS *(25 BC – 50 AD)*

GALEN *(130-200 d.C.)*

The acute inflammatory response & its ideal outcome

Recchiuti et al., 2019 Front. Pharmacol., https://doi.org/10.3389/fphar.2019.00252

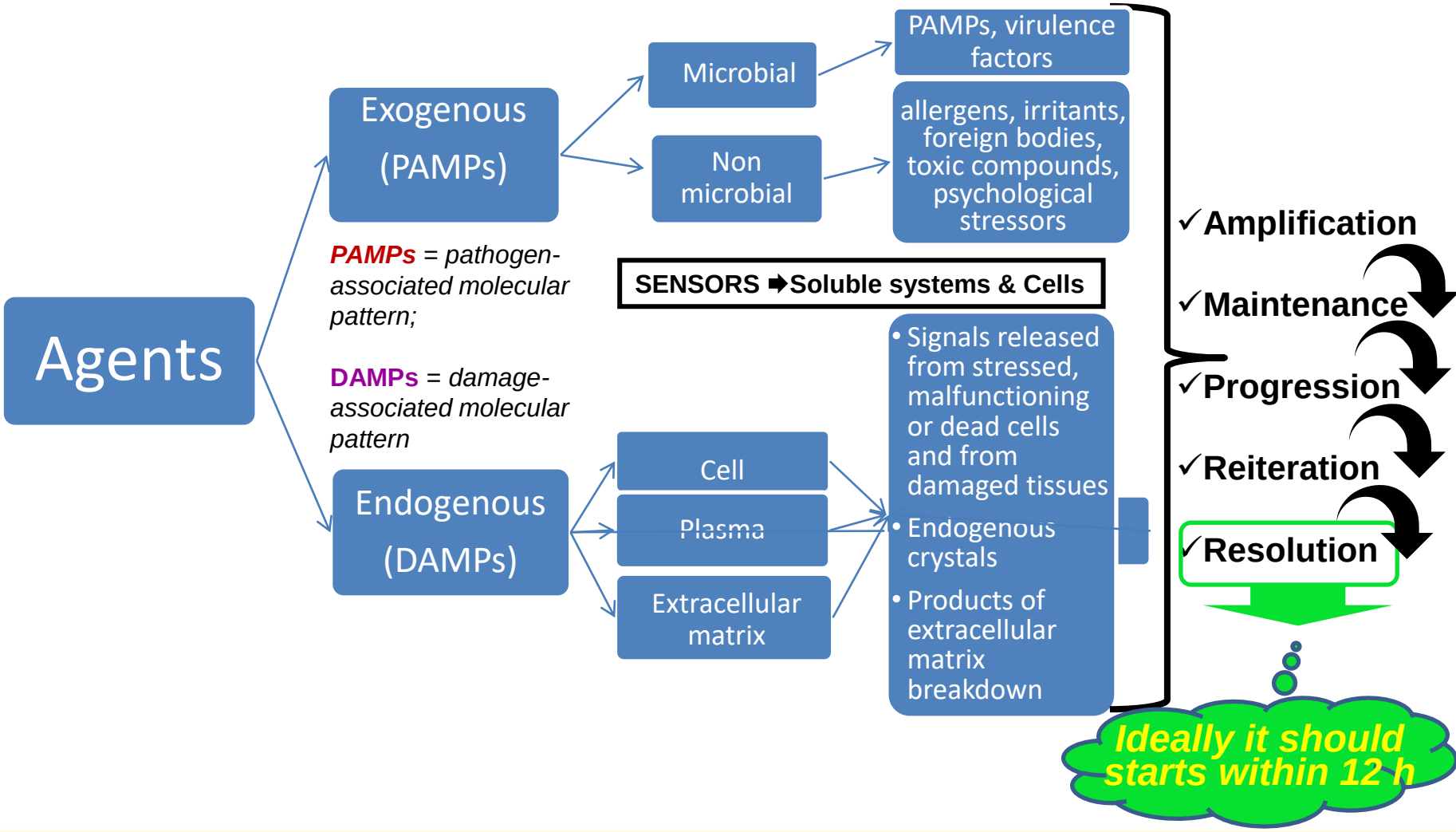


Injury, infections, or dysregulated homeostasis ignites the acute inflammatory response that is normally a host protective mechanism. 1st event in acute inflammation is edema formation, 2nd is infiltration of PMN; 3rd is monocyte-macrophages that clear PMN leading to resolution (essential for ensuring host protection and sparing from tissue damage)

Inflammation: causes

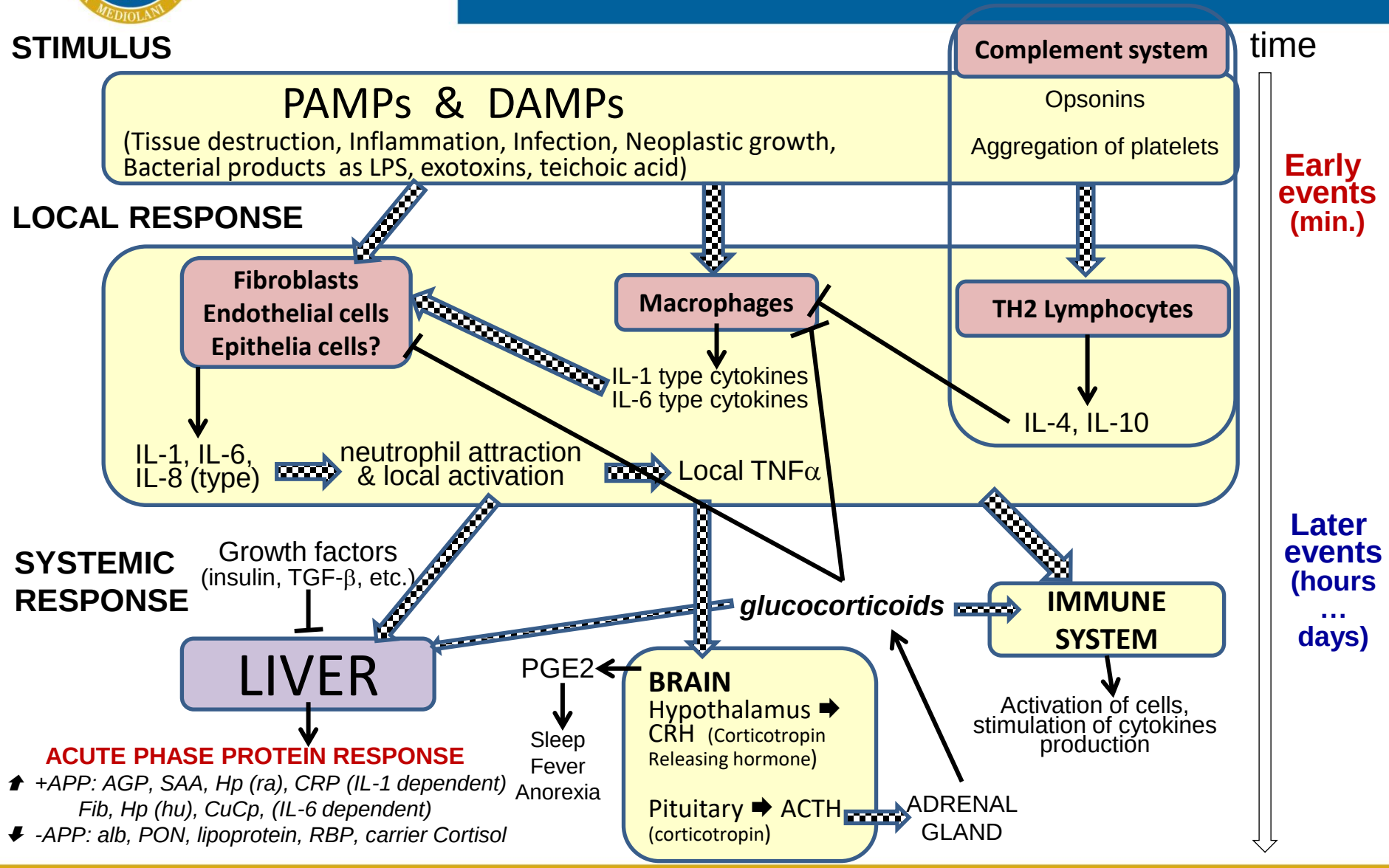
(Medzhitov et al, 2008, modified)

INDUCERS ➡ SENSORS (i.e. TLRs) ➡ SIGNALS (mediators) ➡ EFFECTORS



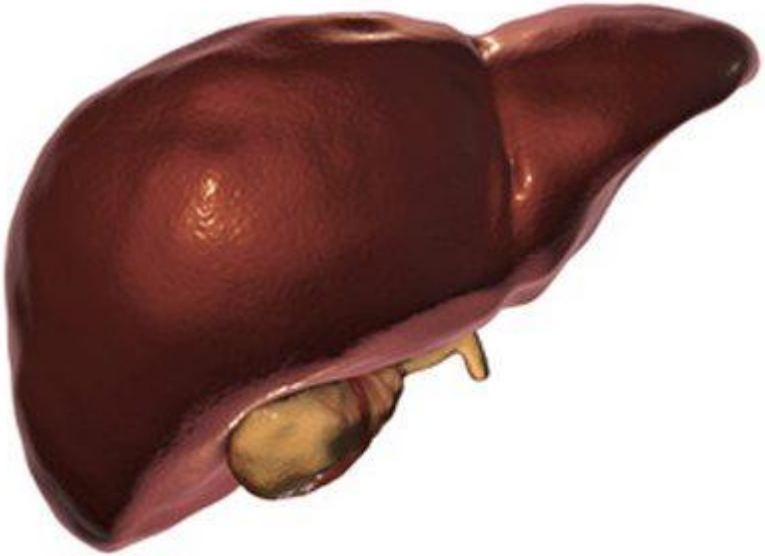
Local & Systemic responses

(modified from Petersen, 2004)



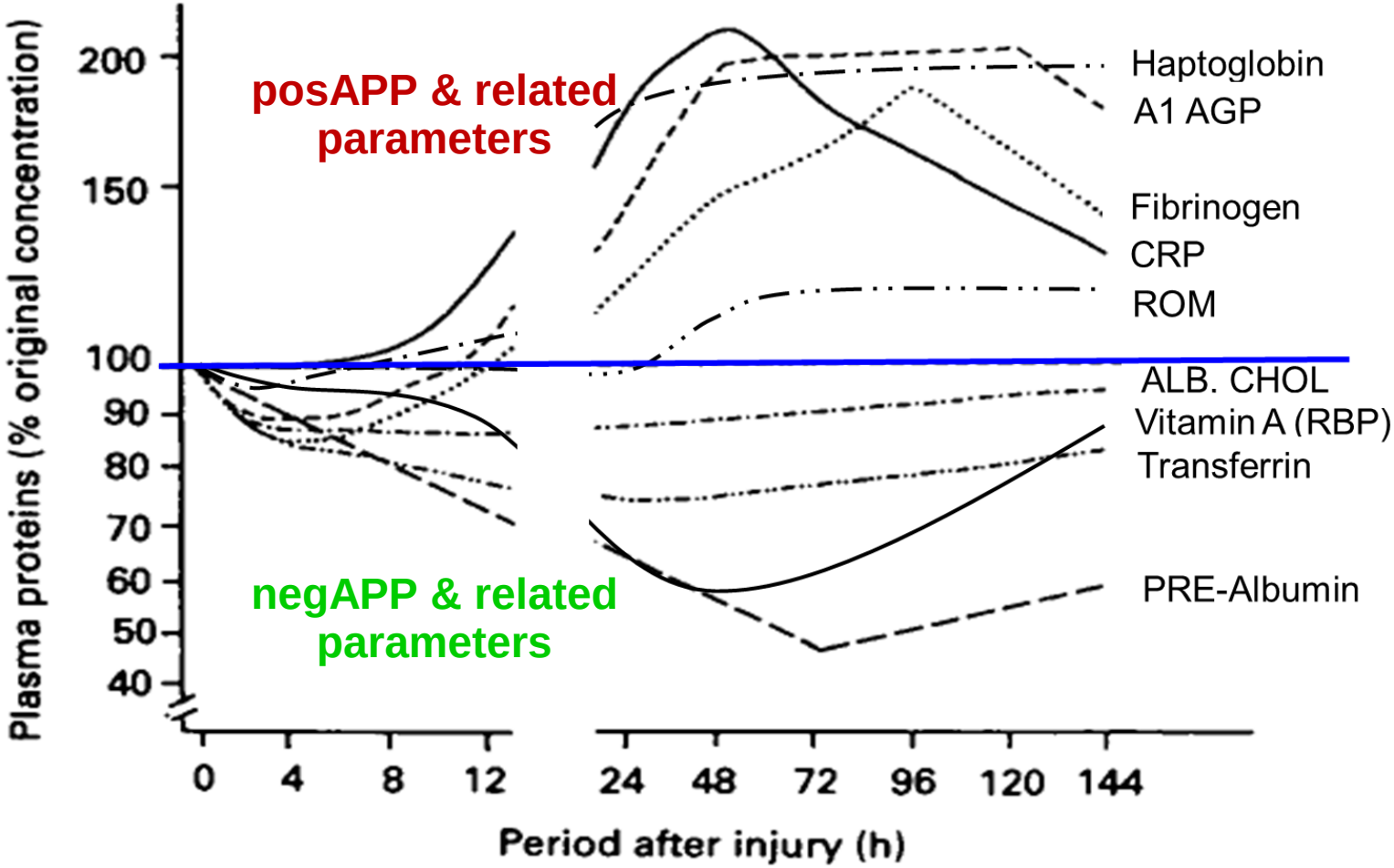
LIVER:
Crucial & conflicting role

- ✓ ***Liver plays a central role*** *for many metabolic pathways*
- ✓ *In case of inflammation, it promotes also the* ***acute phase reaction***
- ✓ *Some pathways in the hepatocytes are:*
  - ***activated:*** ⬆ *synthesis of protective proteins (**posAPP**) and "capturing" protein (e.g. of Fe, Zn)*
  - ***impaired:*** ⬇ *synthesis of common functions (less **negAPP**)*



but the liver is not damaged
(in the short time)

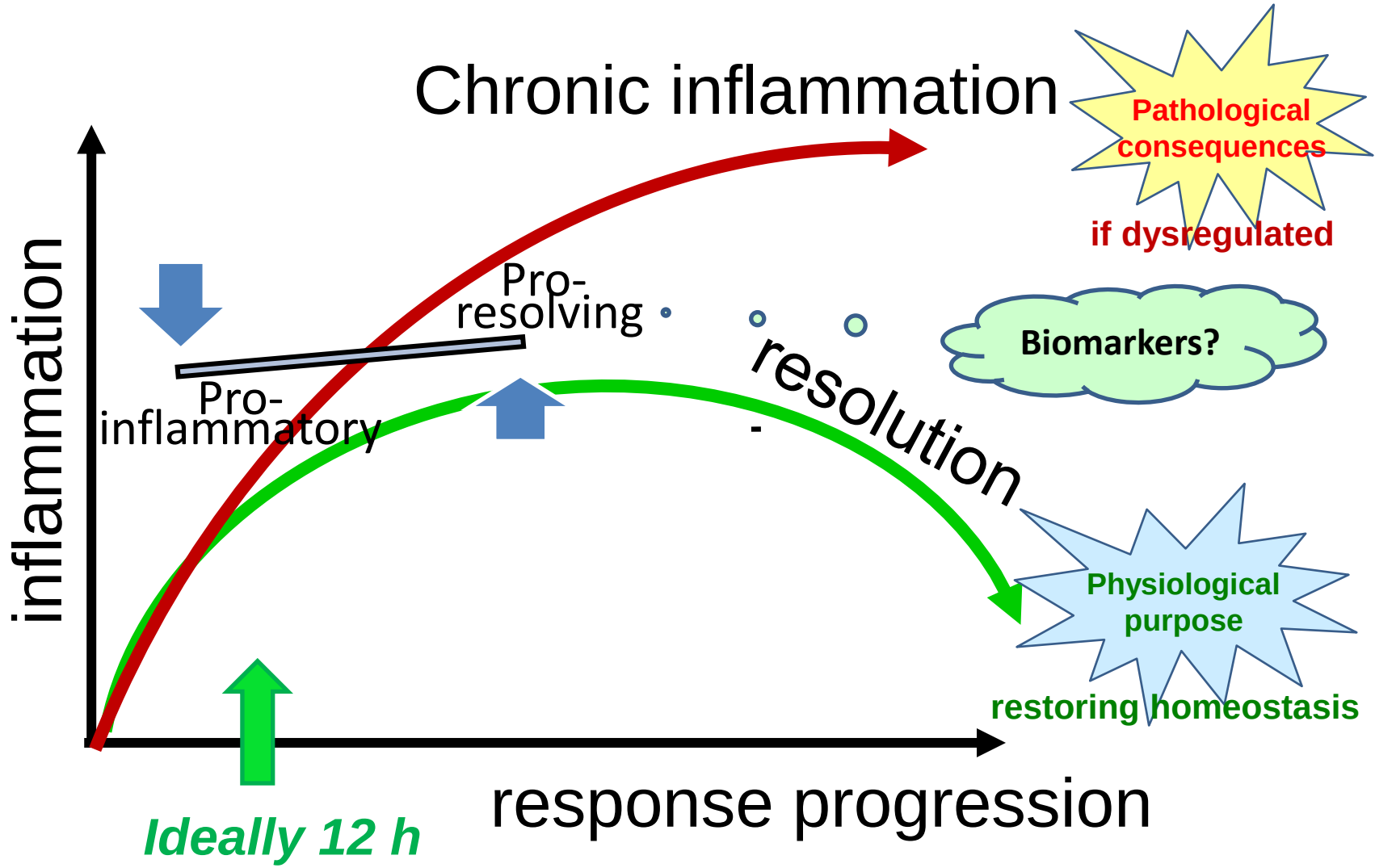
Acute Phase Response



Changes in concentration of plasma proteins after injury or inflammation.

A1 AGP, alpha-acid glycoprotein; CRP, C-reactive protein; ALB, albumin; ROM, reactive oxygen metabolites; CHOL, cholesterol. Changes in CRP and haptoglobin concentrations are plotted on a logarithmic scale (adapted from Fleck, 1989 and Bertoni & Trevisi, 2013).

Inflammation: from physiology to pathology



Summary

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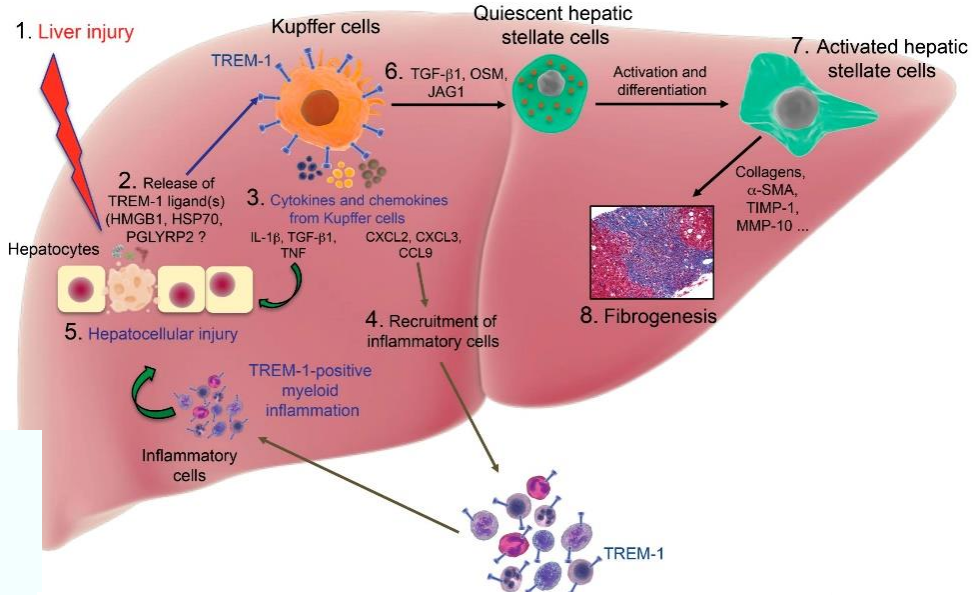
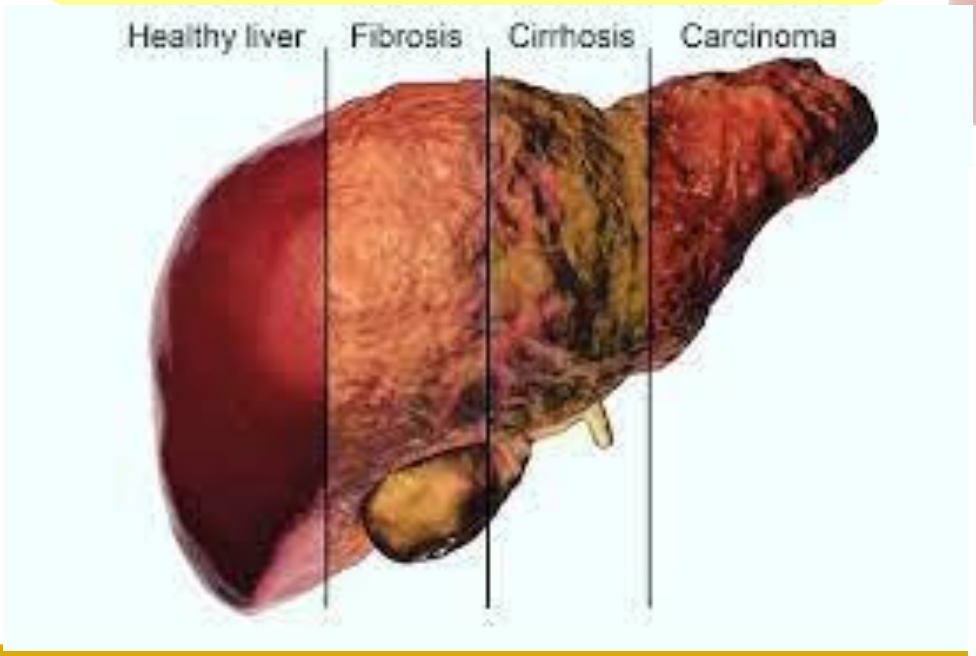
LIVER: Crucial & conflicting role

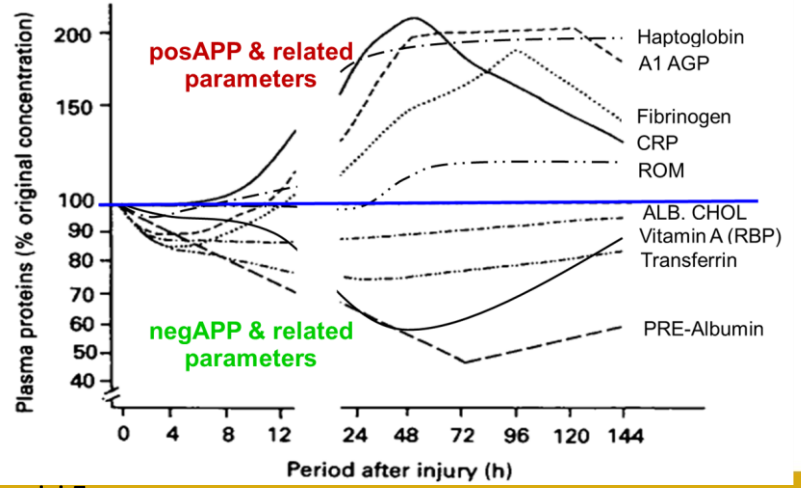
CHRONIC INFLAMMATION in the liver causes FIBROSIS

- ✓Injury activates **Triggering Receptor Expressed on Myeloid cells 1** (TREM-1), which:
- ✓Amplifies hepatic inflammation,
- ✓Activates hepatic stellate cells
- ✓Promotes liver disease & fibrosis

Nguyen-Lefebvre et al., 2018. J Clin Invest. 128(11):4870–4883.







After calving posAPP increased also in healthy cows

Inflammation is not a negative process in itself!
It causes concerns when:

1. Excessive or uncontrolled
2. Reiterated
3. **Resolution is delayed**

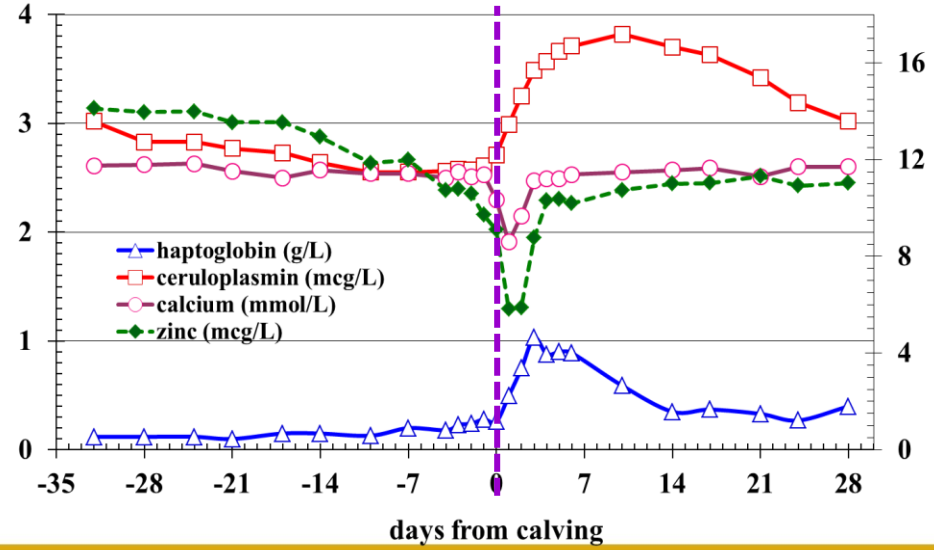
How to assess the severity of an inflammatory event in TP?

Inflammatory presence (i.e. posAPP)?

Inflammatory consequences (i.e. negAPP)?

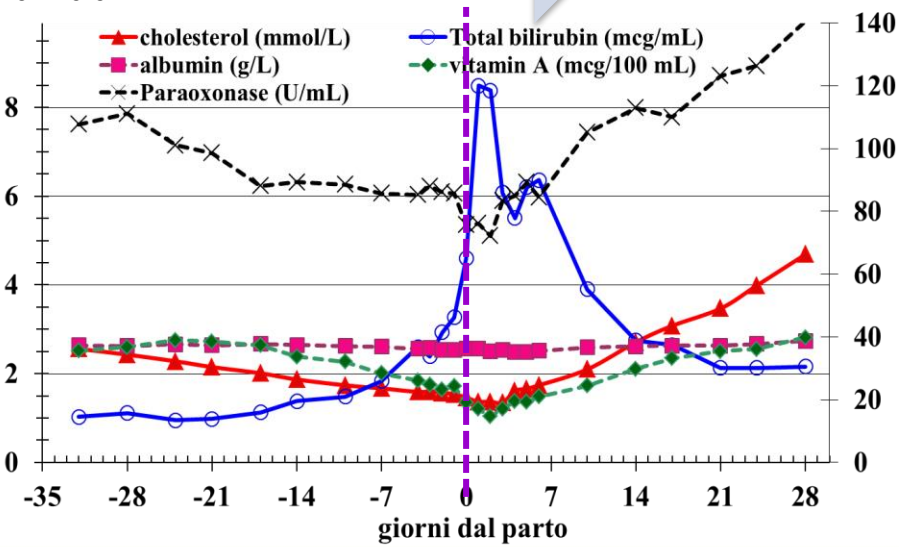
Ceruloplasmin & haptoglobin

Zinc



Cholesterol & bilirubin

…bumin, Vitamin A, paraoxonase



Response to Inflammation: posAPP & negAPP

Bertoni e Trevisi, 2013 VET CLIN N AM-FOOD A 29(2):413–431

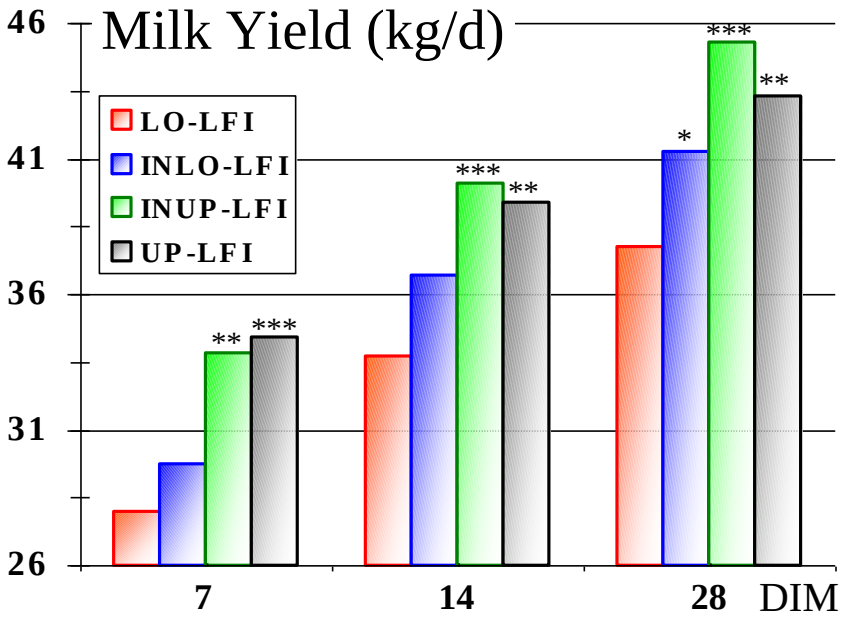
⬆ plasma posAPP

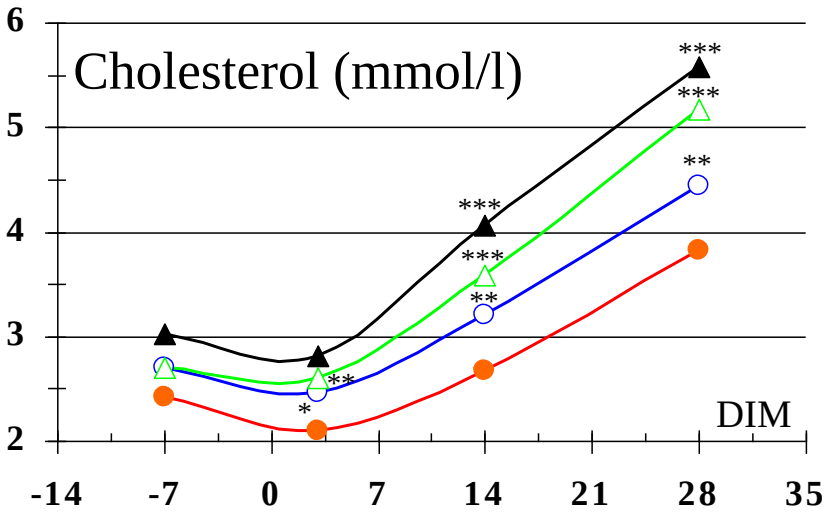
- Positive APP (i.e. haptoglobin, ceruloplasmin, SAA)
- Related parameters (i.e. ROM)

⬇ plasma negAPP

- Negative APP (i.e. albumin, PON)
- Related parameters (i.e. lipoproteins, RBP, total bilirubin)

The post-calving changes of negAPP (i.e. LAI & LFI) resulted more correlated with performance, health, & feed efficiency than posAPP



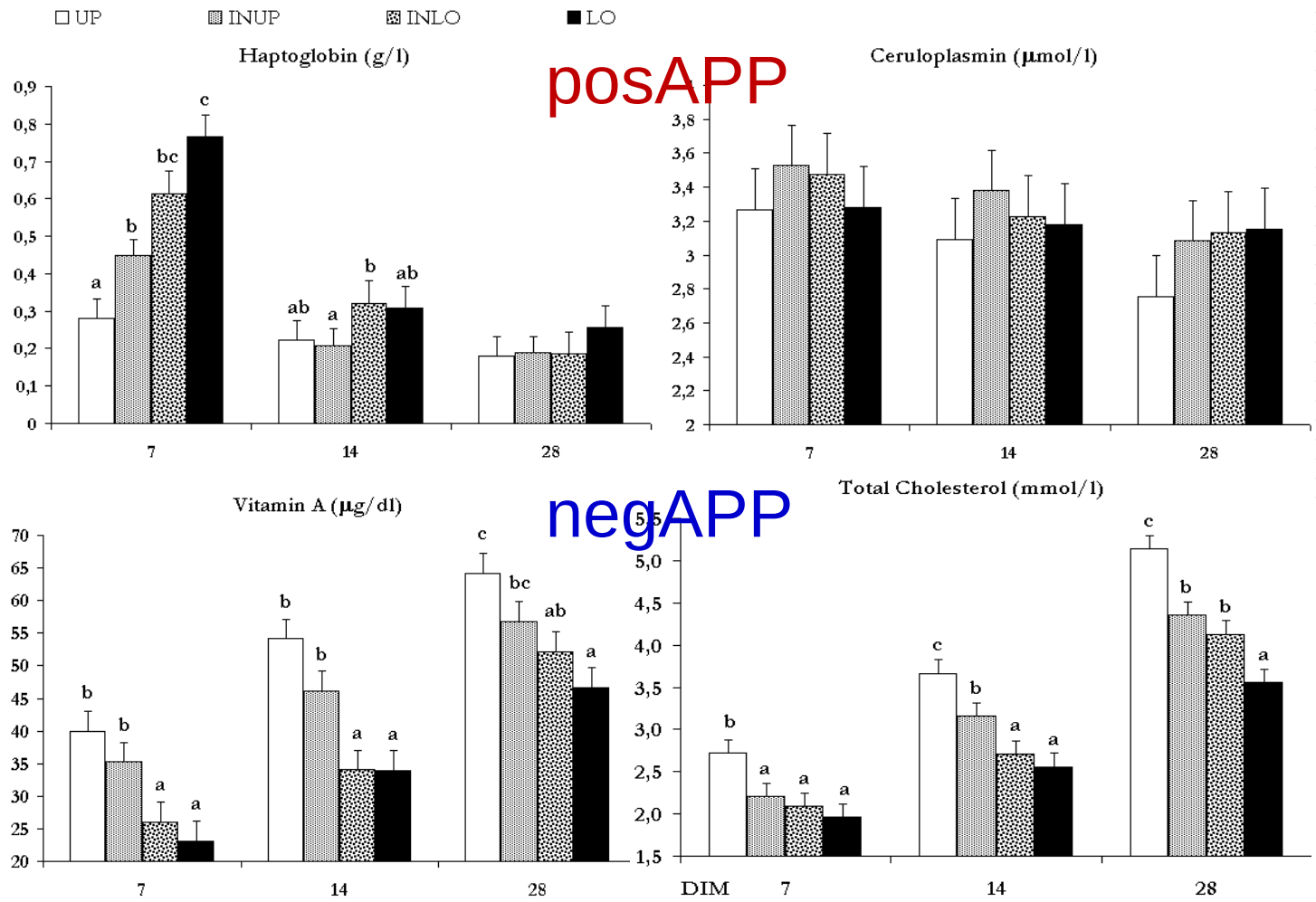


Bertoni et al., 2006, 57th EAAP Meeting, 17-20 Sept., Antalya, Turkey, 325

77 dairy cows ranked in quartiles of Liver Activity Index (LAI)

(Bertoni et al., 2008. JDS 91:3300-3310)

Performances (i.e. peak of lactation, health status) **are inversely related to the severity of inflammation when detected with LAI** *(which includes negAPP: albumin, RBP, cholesterol)*



LFI = Liver Functionality Index

*Bertoni et al., 2006; Trevisi et al., 2016**

parameters

3 plasma biomarkers routinely synthesized by the liver:

albumin

cholesterol (= lipoproteins)

total bilirubin (= indirect measure of the enzymes needed for its clearance)

calculation

- 2 measurements in the 1st month of lactation (3 and 28 DIM)
- I step: index of each biomarker: for albumin & cholesterol= 50% VI + 50% I; for bilirubin = 67% VI + 33% R (VI = value at 3 DIM; I = increase from 3 to 28 DIM; R = reduction from 3 to 28 DIM)
- II step: Normalization of indices obtained in the I step, with reference values (X± SD) obtained from healthy cows and good performances (Bertoni et al., 2008)

$$LFI = \frac{albumins\ index - 17{,}71}{1{,}08} + \frac{cholesterol\ index - 2{,}57}{0{,}43} - \frac{bilirubin\ index - 6{,}08}{2{,}17}$$

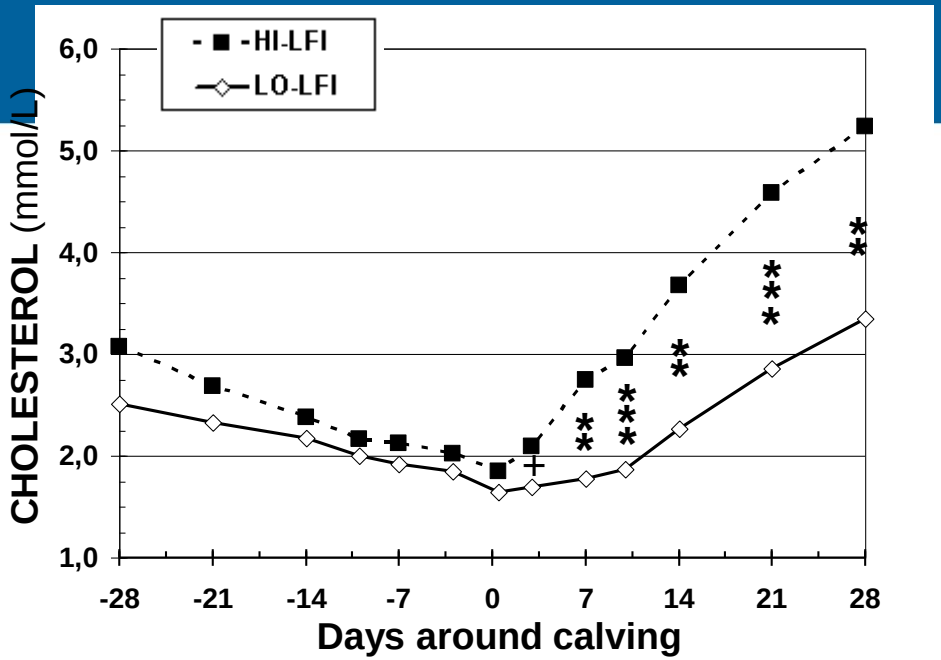
score

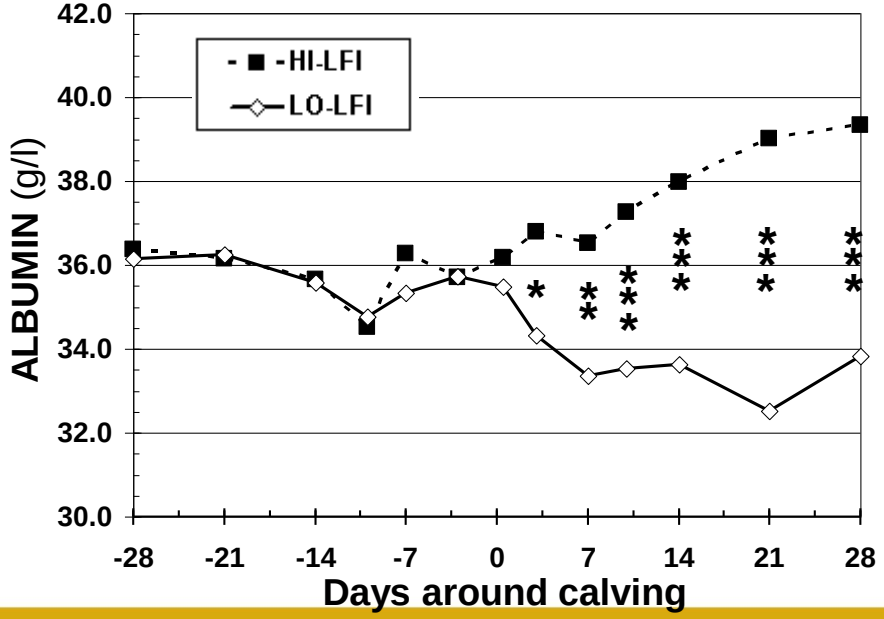
- Final score of each cow is the sum of the normalized indices
- The value can be compared with all the cows (across herds and lactations)
- More is high the value better is the adaptation and low are consequences due to inflammation

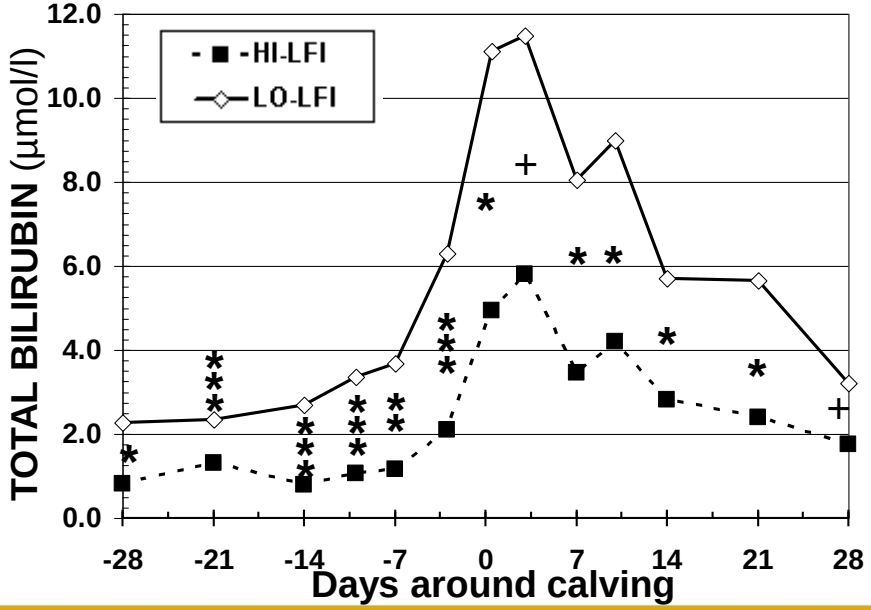
* Trevisi et al. 2006. In "The Innate Immune Response to Non-infectious Stressors" Ed by M. Amadori, Elsevier Inc., The Netherland. Pp 209-235. (ISBN: 978-0-12-801968-9).

LFI = good vs bad response in TP

Direct (albumin, cholesterol) and indirect (bilirubin) indices of negAPP response in plasma samples of periparturient cows with high (HILFI) and low (LOLFI) LFI. (*Trevisi et al., 2012 Res Vet Sci 93:695-704*)

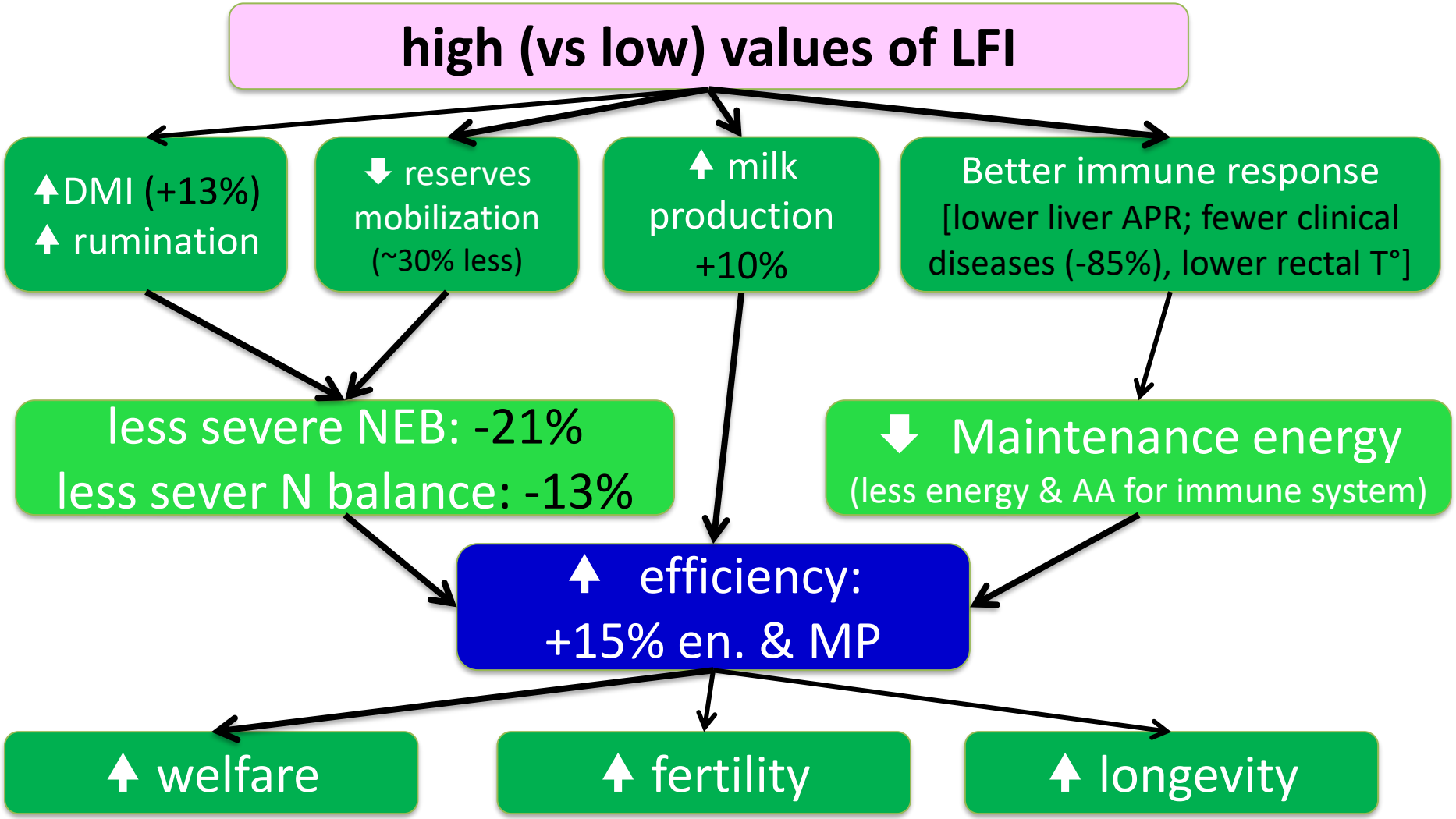






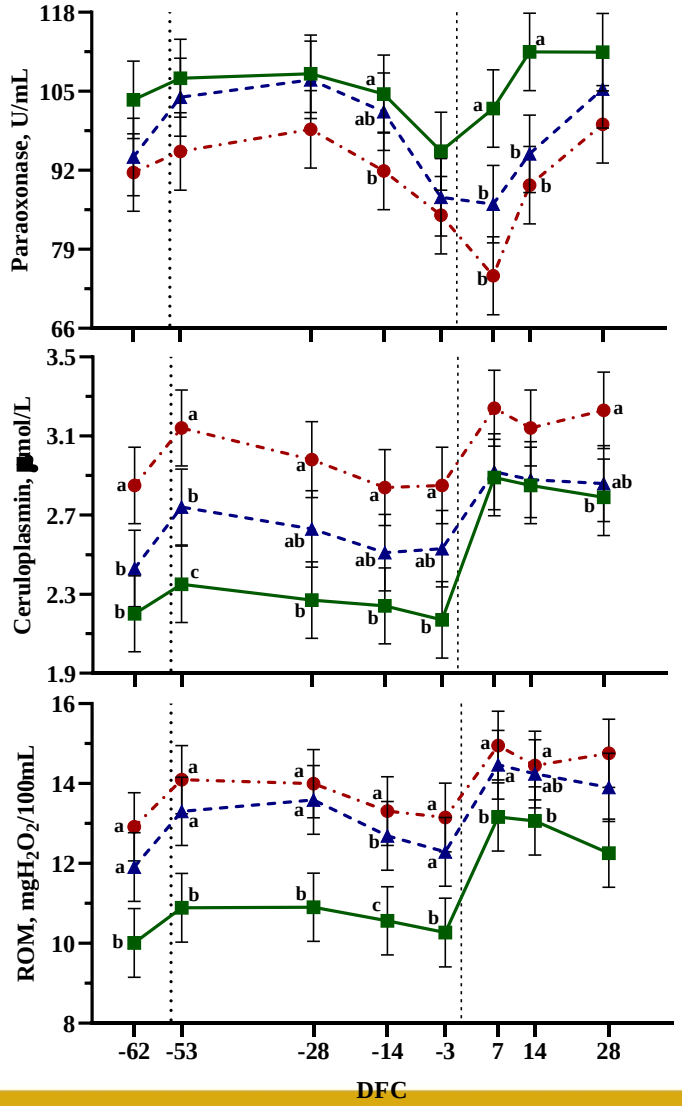
LFI can predict the success of TP (i.e. good vs bad)

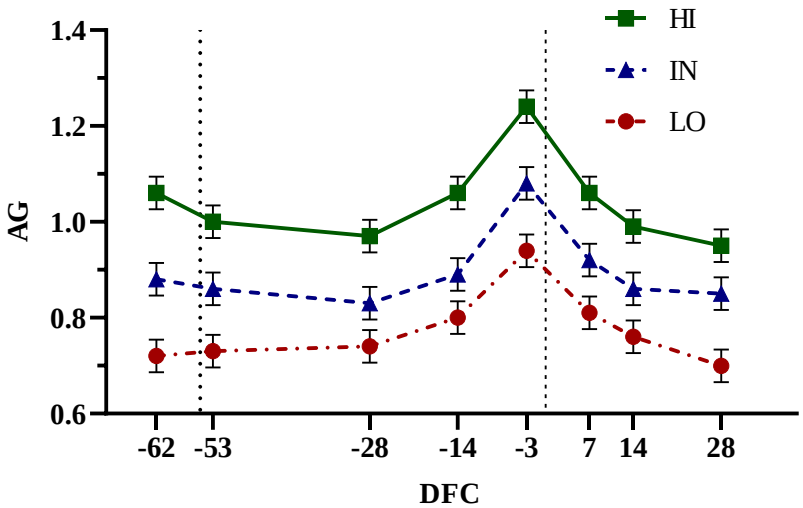
Trevisi et al., 2011 (ISBN 979-953-307-033-8)

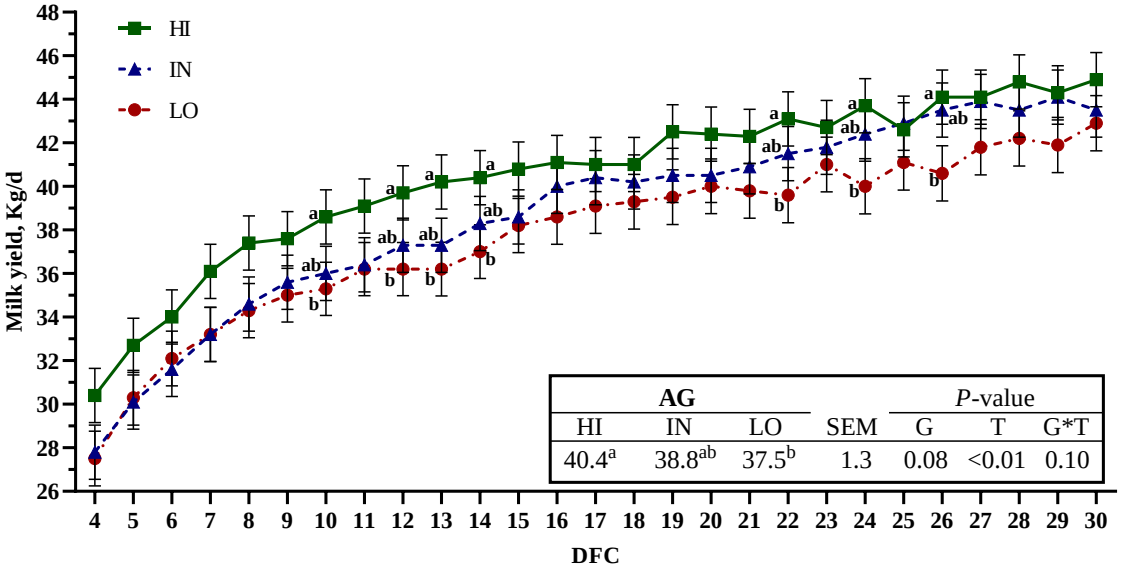


Albumin-to-globulin ratio (AG) before dry-off as a possible index of inflammatory status & performance in the subsequent lactation in dairy cows

(Cattaneo et al, 2021 https://doi.org/10.3168/jds.2020-19944)







AG				*P*-value		
HI	IN	LO	SEM	G	T	G*T
40.4[a]	38.8[ab]	37.5[b]	1.3	0.08	<0.01	0.10

Causes of inflammation in TP
(from mild inflammation to disease)

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Heat/cold stress

Pathogens: virus, gut dismicrobisms *(acidosis, diarrhea)*, **infectious** (ret. placenta, mastitis, metritis, dermatitis)

Oxidative stress: ⬆ metabolism, metabolic diseases (ketosis, displ. abomasum), dry off

Parasites

Activity excess: Dystocia,

Traumas at calving / twin births

Emotional stress (regrouping, isolation, hierarchy, overcrowding, relocation)

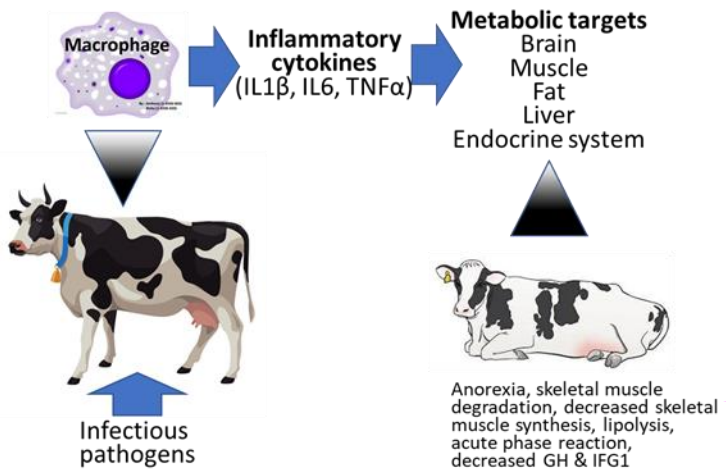
distress
⬇⬆
Inflammation
(PIC, e.g. IL1β, IL6, TNFα)

Nutrition:
Dry: Deficiency/excesses
Early Lact: Deficiency, Micotoxins, digestive disorders,

Genome

Physical symptoms:
fever, tissue damage, pain

Mental symptoms
loss of interest for environment, depression, irritability, mild cognitive disorders, suffering
(Dantzer et al., 2008)



Adapted from Johnson & Finck (2001)

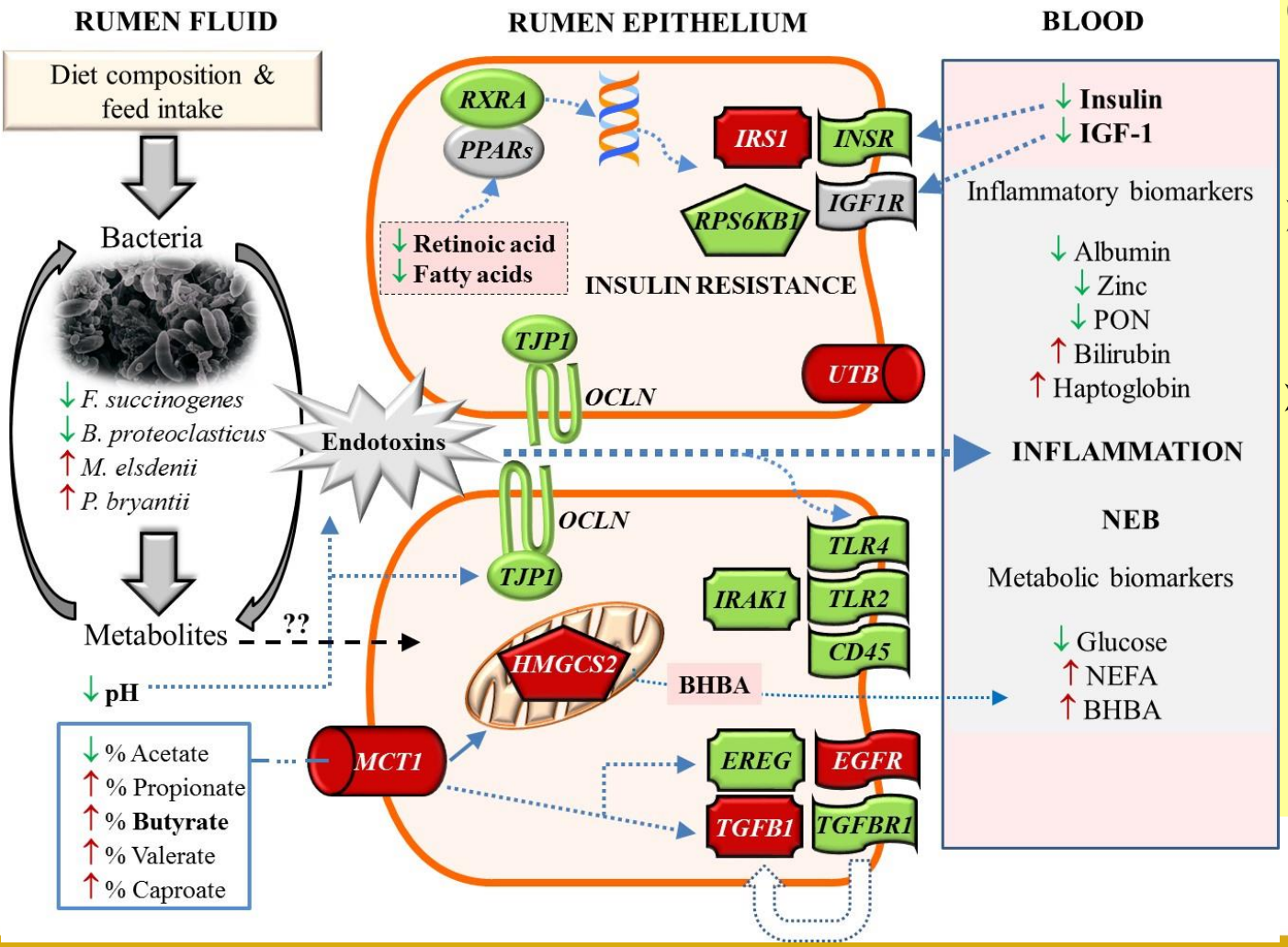
PAMPs *= pathogen-associated molecular pattern;*
DAMPs *= damage-associated molecular pattern*

Many are sneaky

Changes of rumen physiology in TP

Minuti et al., *JDS* 2015, 98:8940-8951

Down-reg.
Up-reg.



Gene expression (GE) in rumen epithelium, Post vs Pre - partum:

- ↓ GE of tight junction
  ↓
  ↑ Permeability
- ↓ GE of many immune functions
  ↓
  ↓ Epithelium protection
- ↓ GE of glucose uptake
  ↓
  Insulin resistance

Energy concentration in dry period & DMI – health status

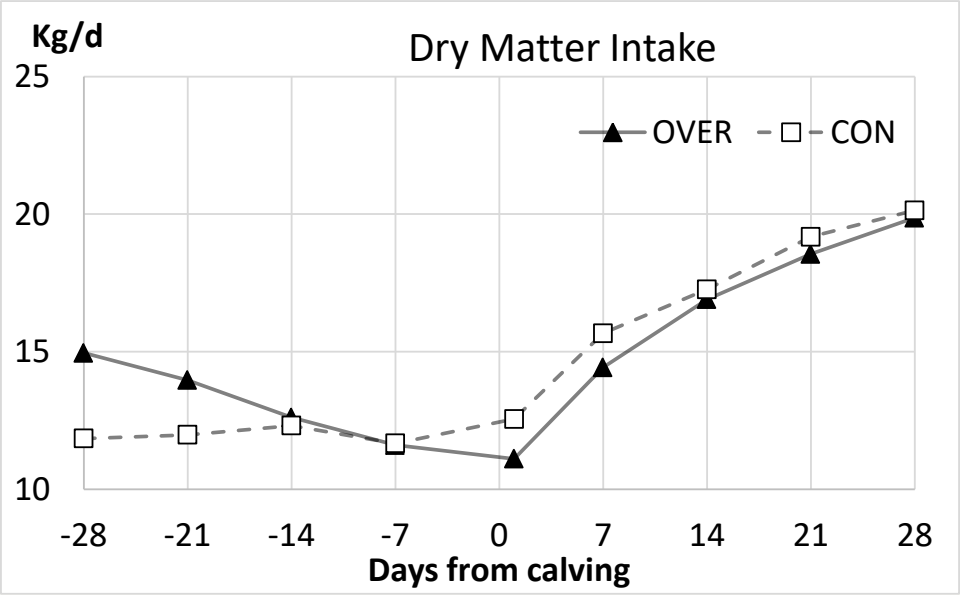
Janovick et al. 2011. JDS 94 :1385–1400

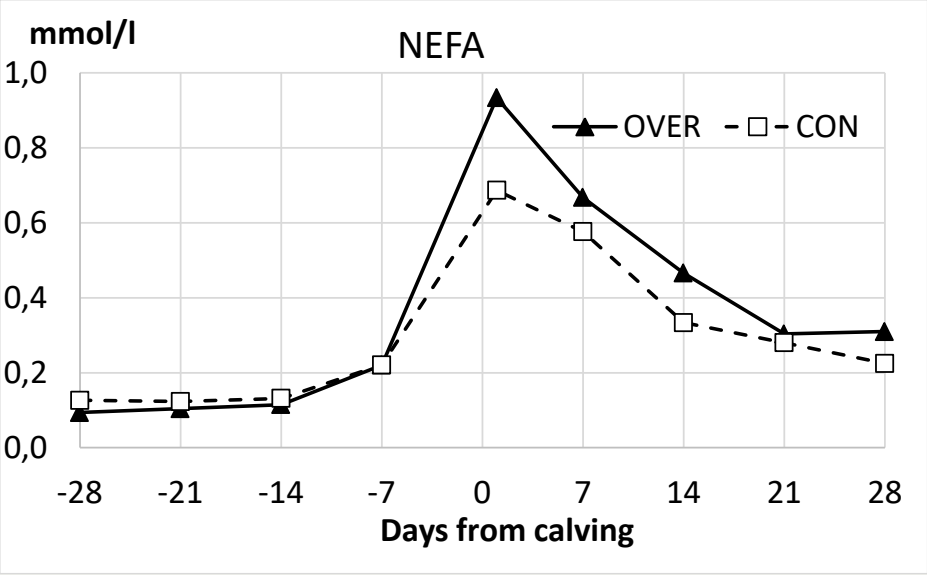
		CON	OVER
% energy requirement		**100**	**150**
NE_L	Mcal/kg	1,26	1,61
CP	% DM	15,00	15,75
Starch	% DM	13,2	21,4
NDF	% DM	49,20	38,35
ADF	% DM	33,25	26,30
Ash	% DM	10,9	8,4

	Health problems (% of total problems)							
	Cow (n)	Clinically ill cows (% of cow) (1)	Abomasal displacement	Retained placenta	Dystocia	Metritis	Mastitis	Clinical Ketosis
OVER	39	**62,1**	**20,7**	**37,9**	**3,4**	**13,8**	**24,1**	**37,9**
CON	28	**32,1**	**10,7**	**10,7**	**0,0**	**10,7**	**10,7**	**10,7**

(1) cows with at least one clinical health problem

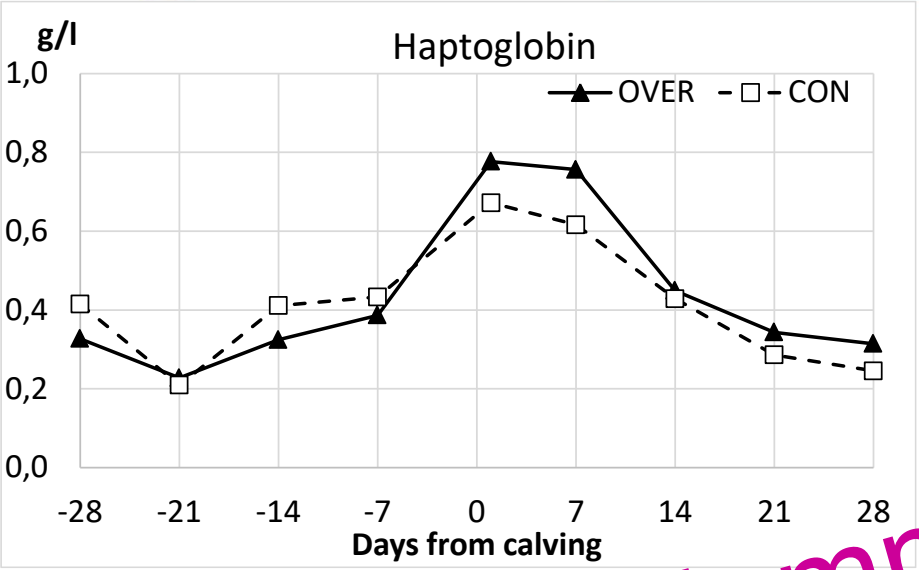
OVER mobilize more body reserves (BCS -28/+28 DIM: -0.85 vs -0.69 points)

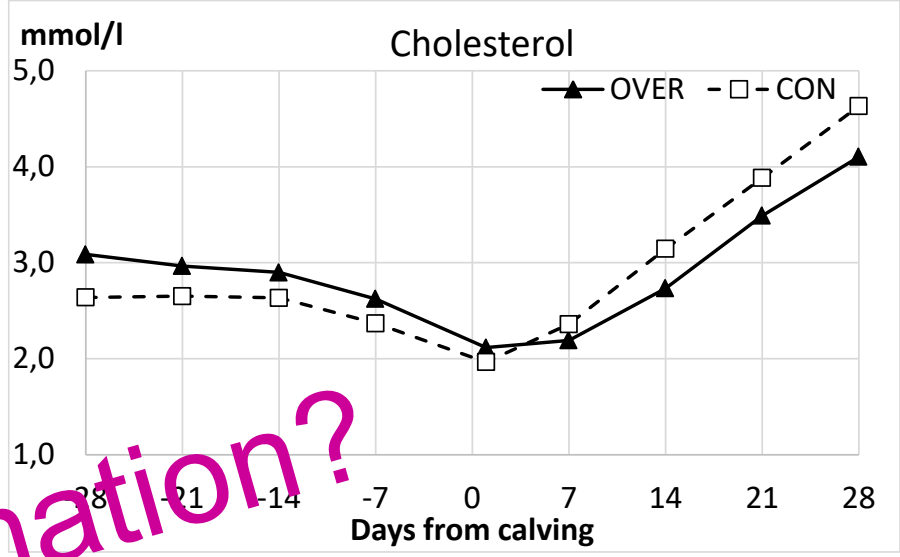


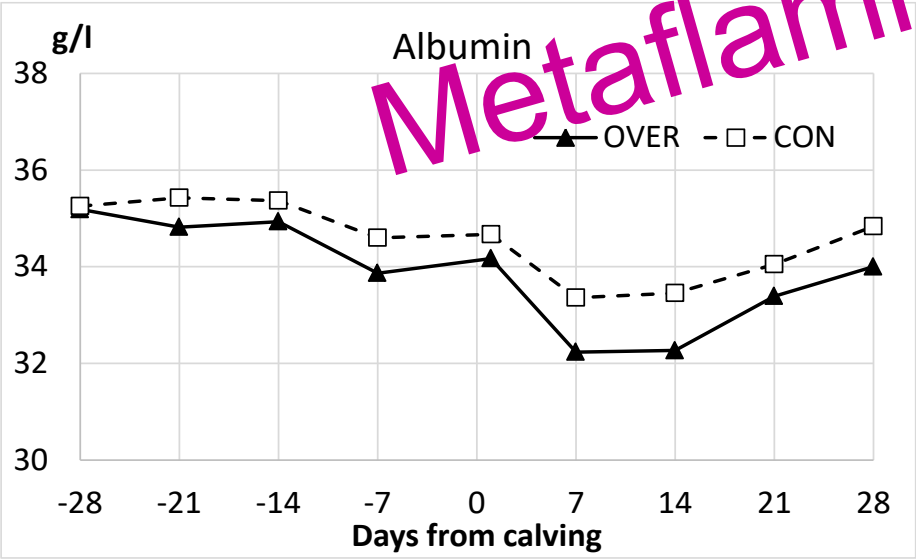


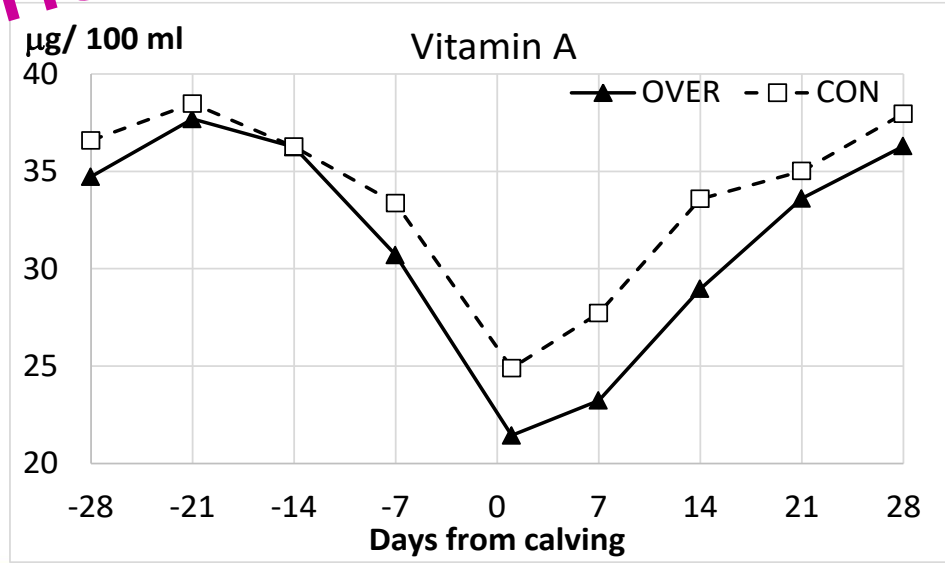
Energy concentration in dry period & APPs

(some changes tend to start before calving)





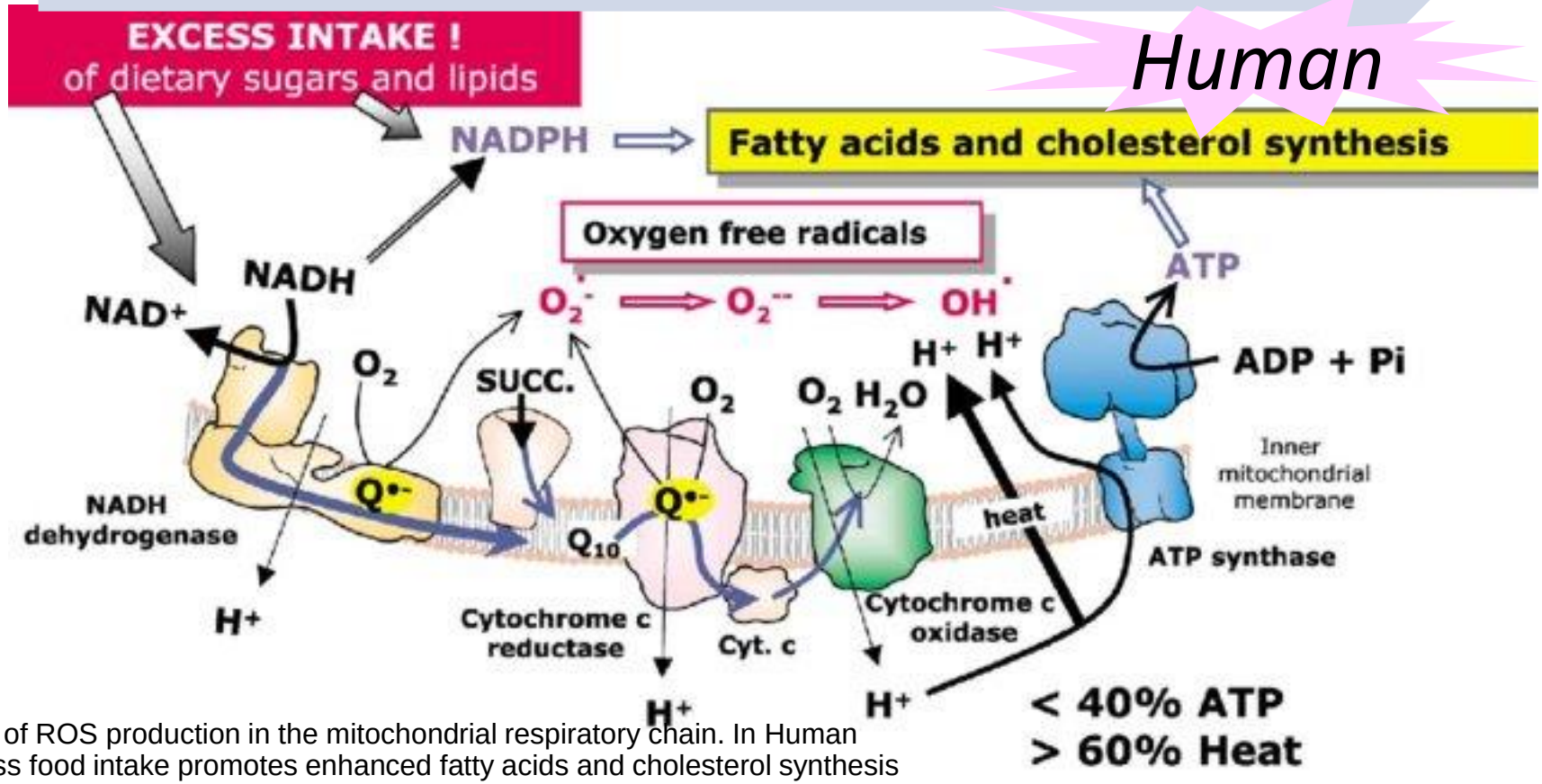




Metaflammation?

OS, intake of energy, inflammation/metainflammation

Excessive energy intake → ⬆ release of ROMs (and heat) → Inflammation

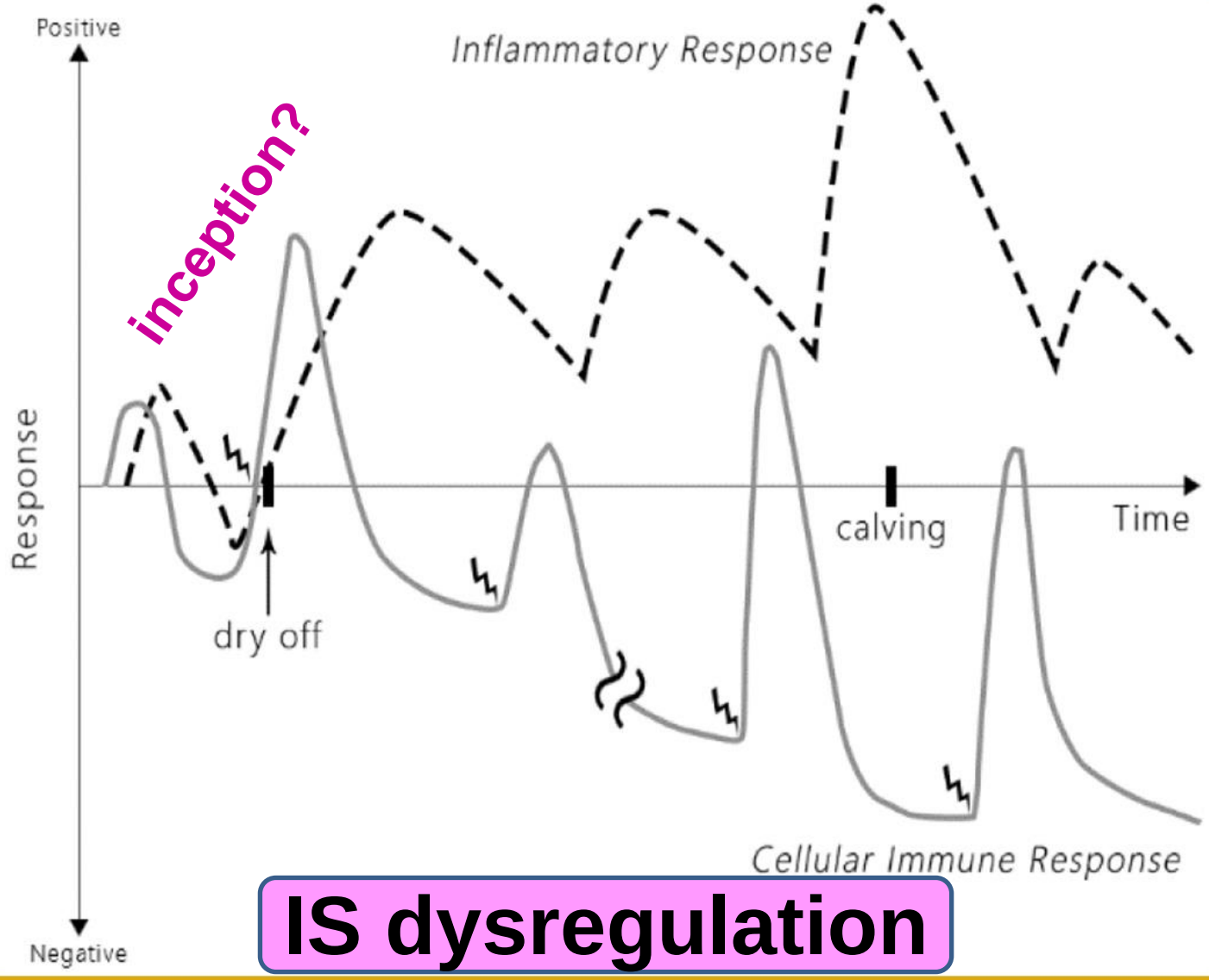


Sites of ROS production in the mitochondrial respiratory chain. In Human excess food intake promotes enhanced fatty acids and cholesterol synthesis and ROS generation by respiratory complexes I and III (Jackson et al., 2002 Molecular Aspects of Medicine 23:209–285).

Hypothesis: can successive inflammatory challenges cause the IS dysregulation in TP?

The possible **relationship** between the **cellular immune response** (——) and the **systemic inflammatory response** (– – –) consequent **to multiple insults that could occur from dry off to calving**.

(Trevisi & Minuti, 2018, modified from Heyland et al., 2006)



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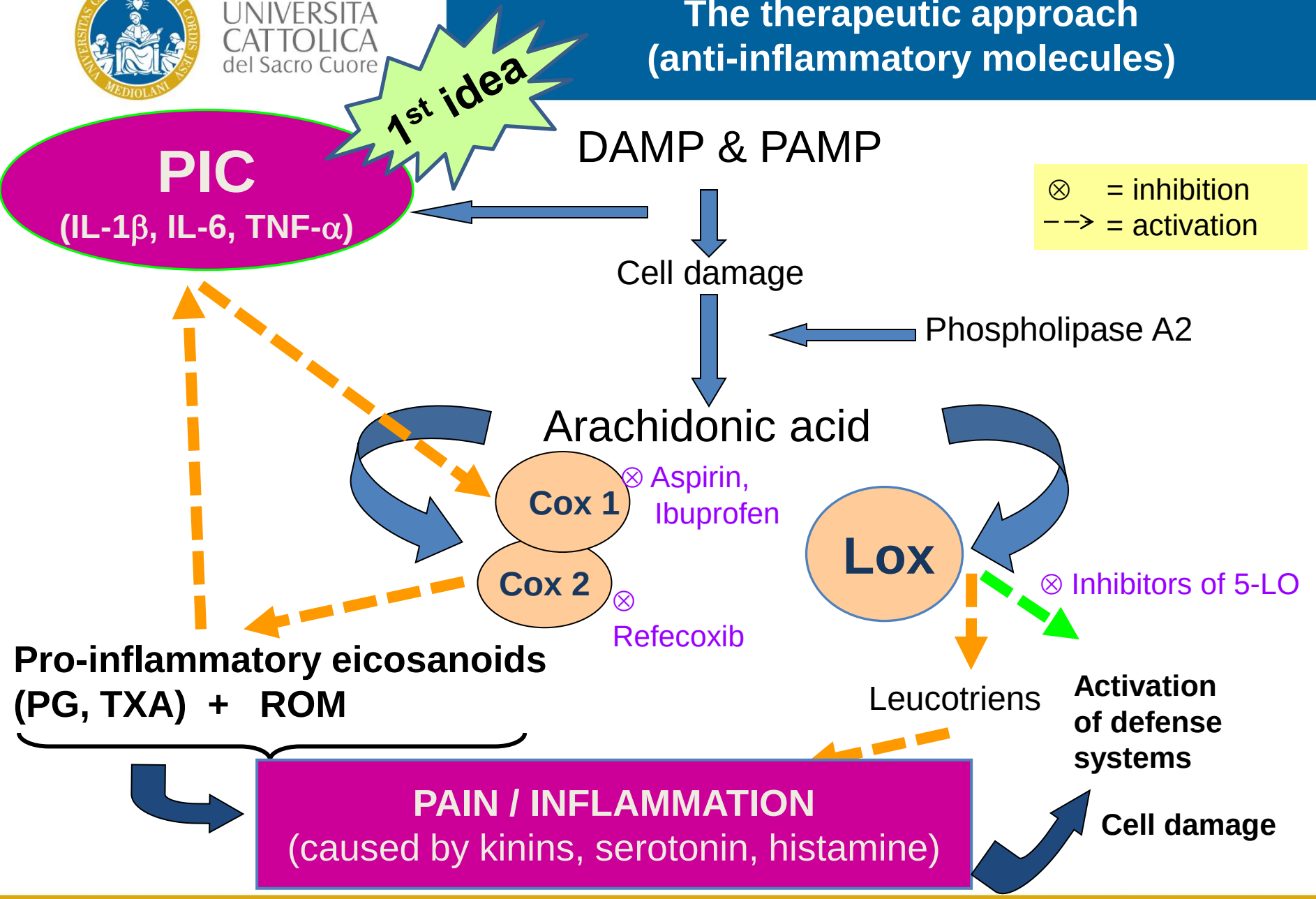
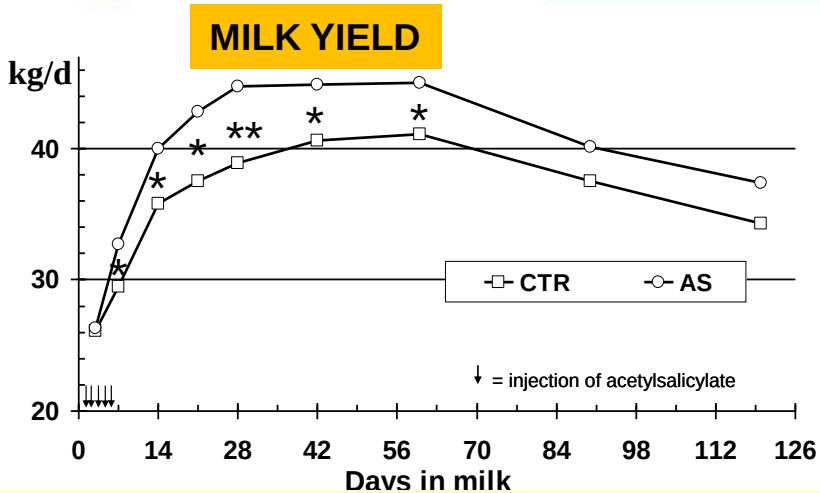
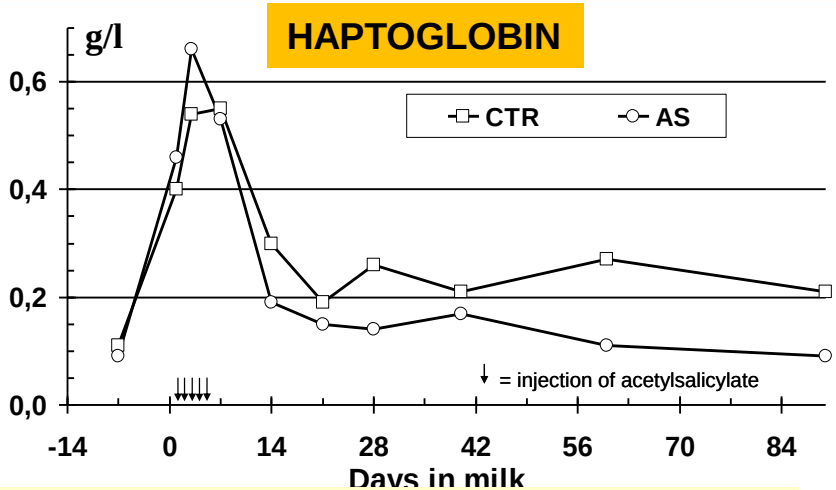
HOW TO ATTENUATE THE INFLAMMATION?
The therapeutic approach (anti-inflammatory molecules)



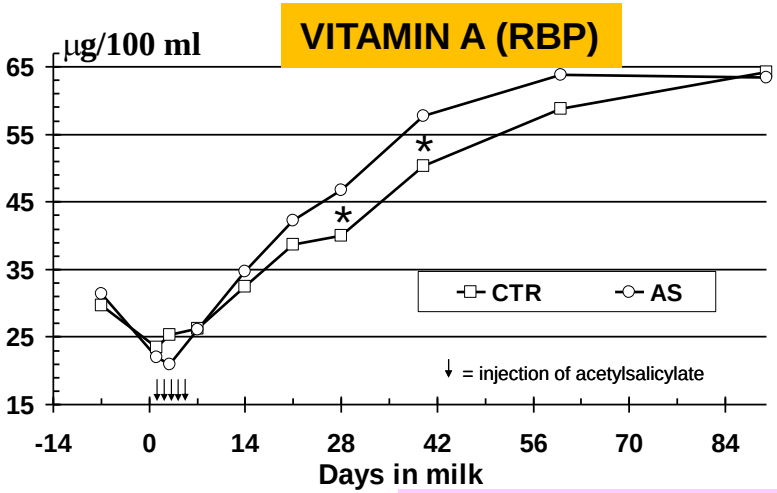
Model "NSAIDs" in TP

Trevisi et al., 2003. 54 EAAP Meeting 8:258 - Bertoni et al 2004 Vet. Res. Comm. 28(S1):217-219 - Trevisi et al., 2008 J. It. Ass. Buiatrics 3:61-6 - Trevisi et al., 2010 14 ICPD Meeting:165-166





AS = **lysine acetylsalicylate** daily i.m. treatments (15 g/d 1,2,3 DIM & 7.5 g/d 4,5 DIM)
CTR = control



FERTILITY group		CTR	AS
cows	*n°*	22,0	23,0
culled cows	*% of total*	15,8	9,5
pregnant cows	*% of total*	86,4	91,3
pregnant at 1st insemination	*% of pregnant*	21,1	52,4
repeat breeders	*% of pregnant*	36,8	28,6
services per pregnancy (§)	*n°*	2,68	2,38
open days (§)	*n°*	131,8	106,3
Fertility Status Index (FSI) (@)	*n°*	12,6	61,8

LEGEND: (§) after logarithmic tranformation; (@) Esslemont and Eddy (1977).

Trevisi e Bertoni (2008) In "Aspirin and health research progress", Nova Science Publishers. pp 23-37

How to act?

The attenuation of inflammation in the TP improves performance, health & welfare of dairy cows (aspirin model)

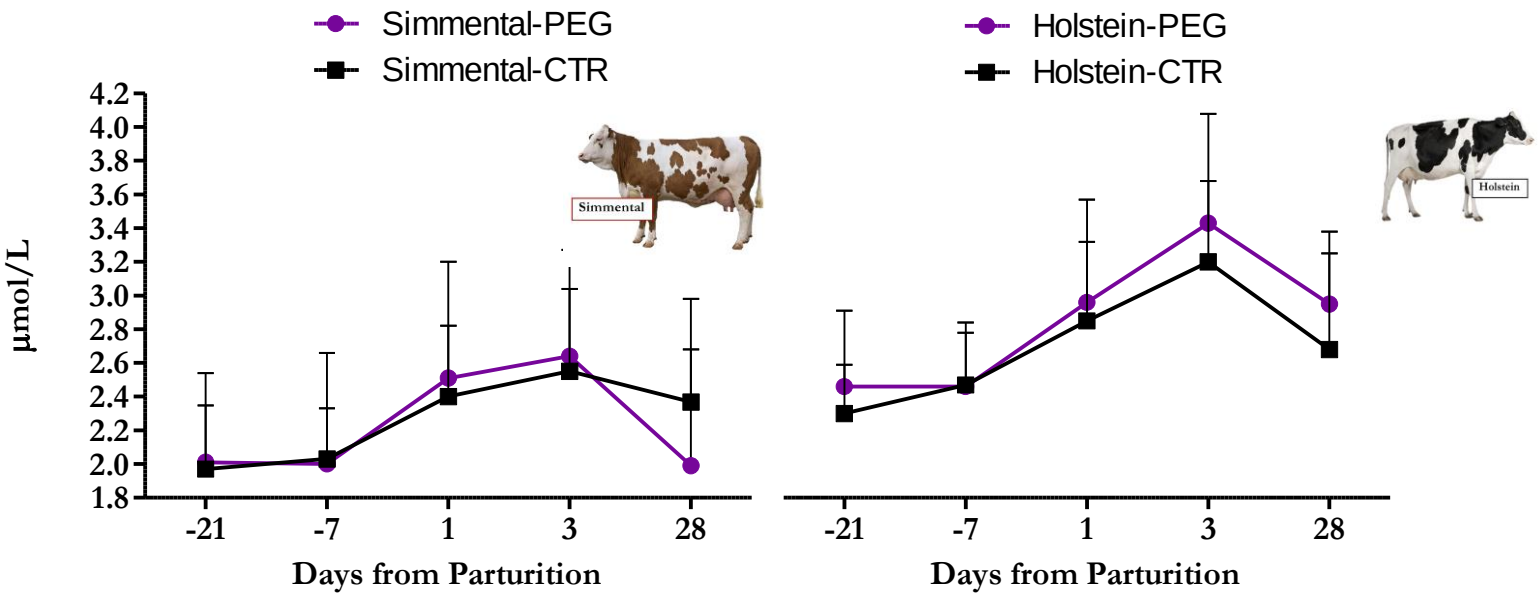
Possible strategies to modulate the inflammation:

1. **Genetic selection** (for optimized immune response)
2. **Immunomodulation** *(e.g. by tuning the immune cells recruitment)* by pharmaceutic treatments
3. **Immunonutrition (nutraceuticals)**: the most effective strategy in the short period *(e.g. balancing ω6 and ω3 ratio, polyphenols, CLA, special β-glucan)*

① Breed effect on posAPP in TP

Lopreiato et al. 2019 JDS 102(10):9312-9327

Pattern of changes of plasma ceruloplasmin in the peripartum



Bred x Treatment x DFP $P = 0.04$

Similar data were also presented by Curone et al. (2018, RVS 116:88-98) comparing breeds with different genetic merit

Genetic regulation of biomarkers as stress proxies in dairy cows

Milanesi et al., 2021 Genes 12(4), 534; https://doi.org/10.3390/genes12040534

- Tested the **association of 21** metabolic, immune response, liver function **biomarkers with genotype** (150K SNPs) of 416 Italian Simmental & 436 Italian Holstein cows
- Carried out a **Single-SNP & haplotype-based genome-wide association studies** (**GWAS**) to assess whether the variation in the levels of 21 biomarkers was under genetic control & to identify the genomic loci involved
- **Found significant associations** for plasma levels of **ceruloplasmin** (BTA1), **PON** (BTA4) and **GGT** (BTA17).
- These biomarkers are primarily produced by the liver and have functions related to protection from oxidative stress
- **Is the level of expression of these biomarkers correlated with the response to stressful situations (as TP)?**

BTA = Bos taurus chromosome

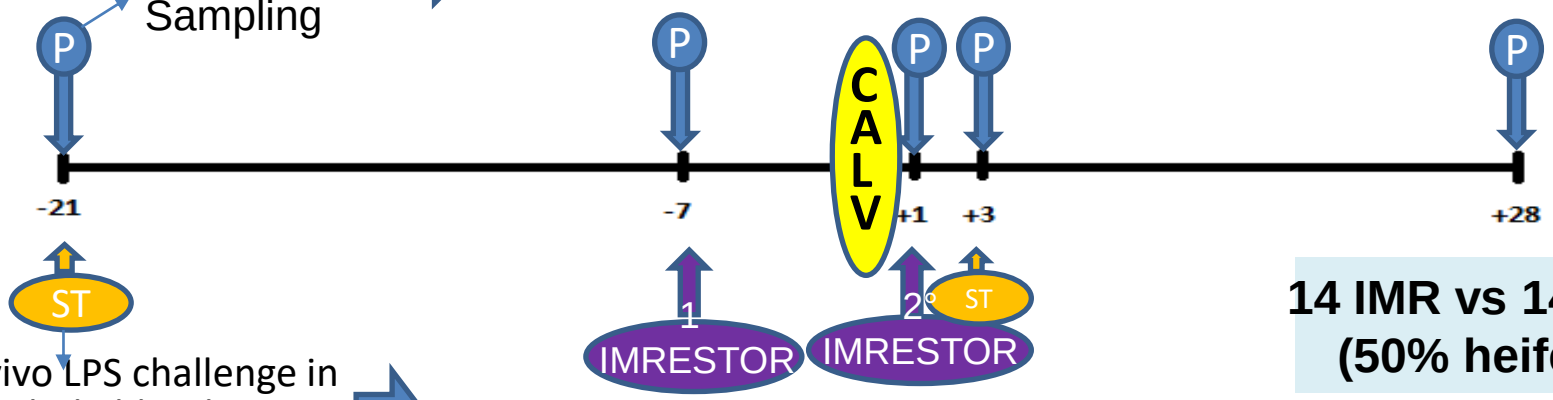
② Pegbovigrastim (rbG-CSF) in TP

Recombinant Bovine Granulocyte Colony-stimulating Factor

Lopreiato et al. 2019 JDS 102(10):9312-9327

Blood Sampling

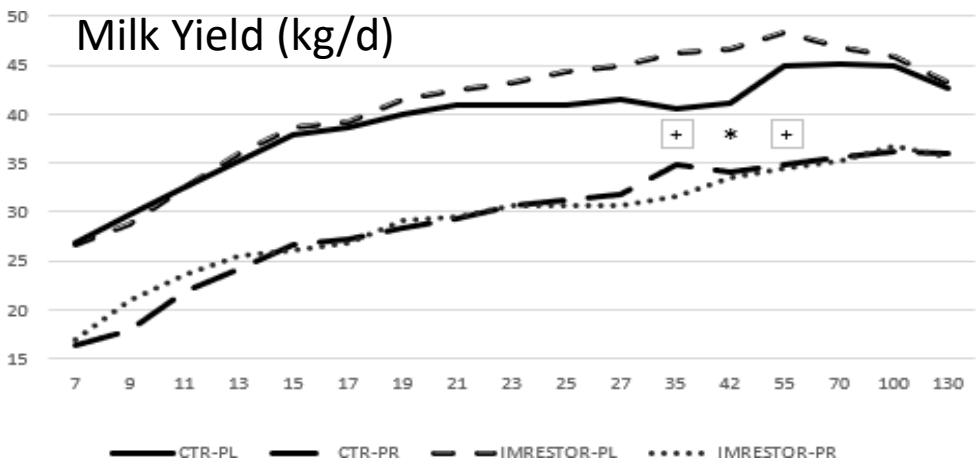
Several blood biomarkers for the metabolic profile assessment

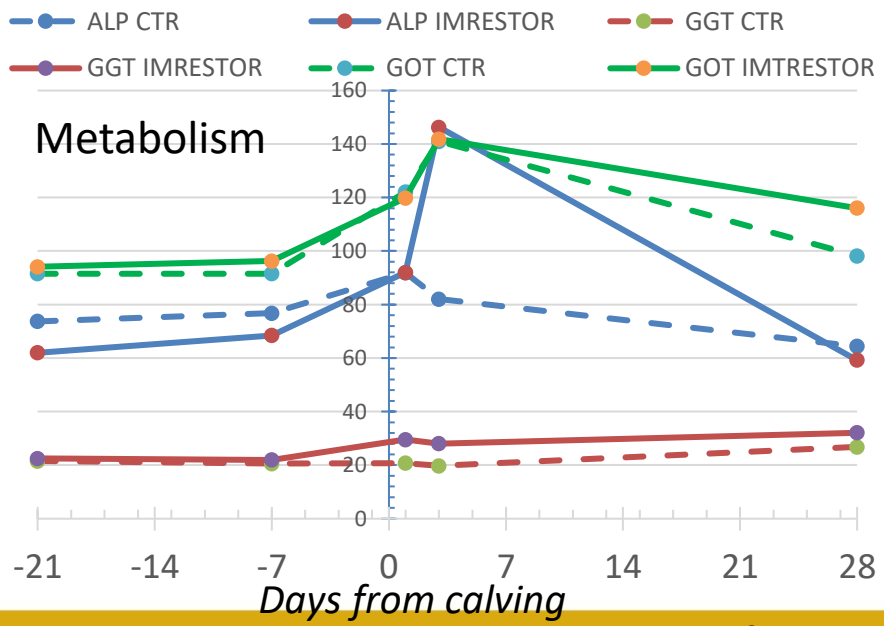


14 IMR vs 14 CTR (50% heifers)

ST: ex-vivo LPS challenge in whole blood

IL-6 e IL-1b Analysis (basal & LPS-challenged)





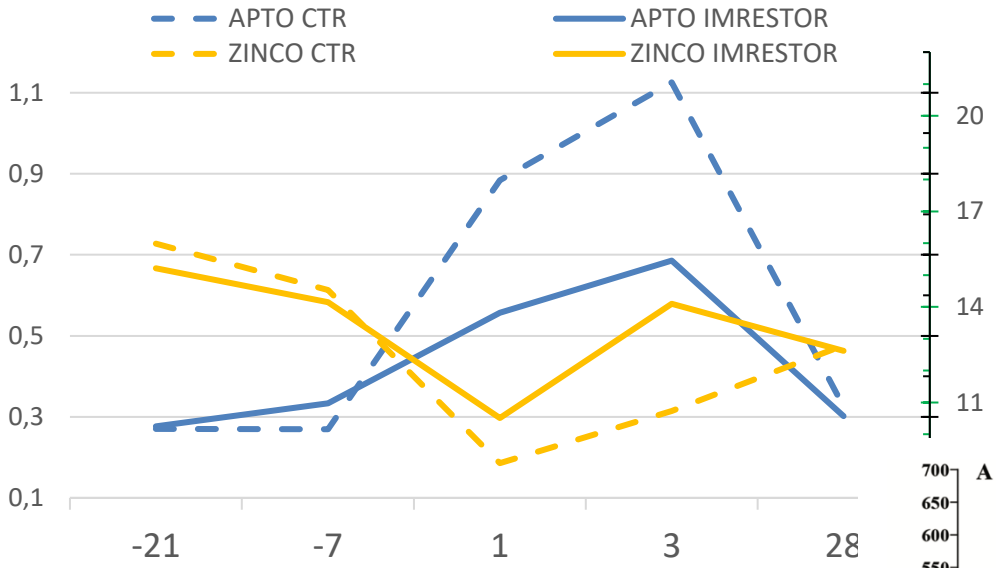
Milk production: ⬆ (*only multiparous*) 250 kg/head/day in the first 130 d of lactation

Pegbovigrastim (rbG-CSF) in TP

Lopreiato et al. 2019 JDS 102(10):9312-9327

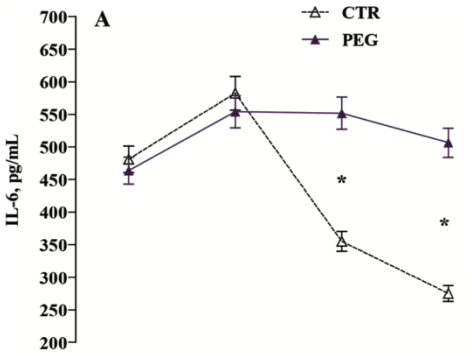
IMR cows: a lower severity of inflammatory response in the post-partum

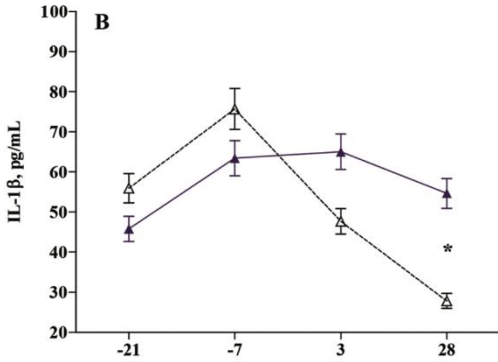
	Stimulated IL6/IL1b	
	-21	3
CONTROL	0,49	0,66
IMRESTOR	0,47	1,06
		**



LPS-challenge: IMR cows showed higher plasma levels of IL6 rather than IL1b.

Faster recovery state?

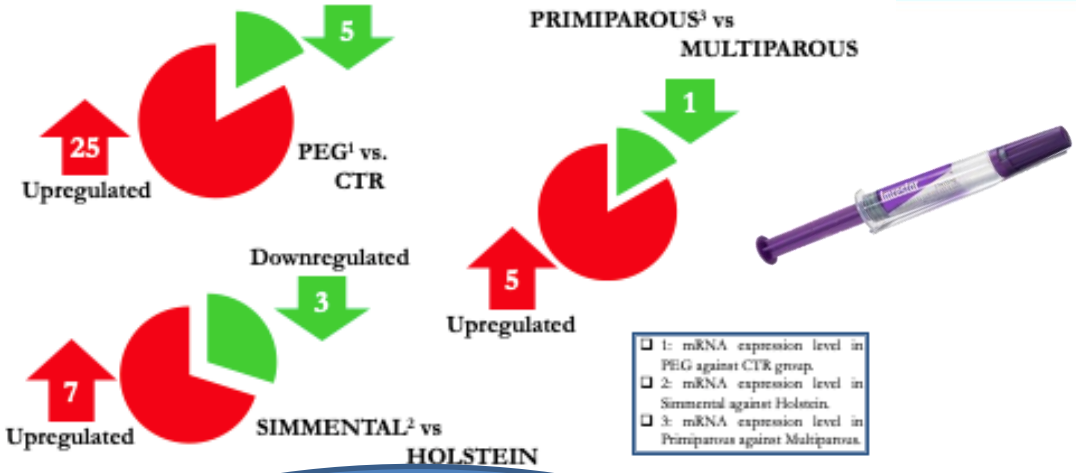


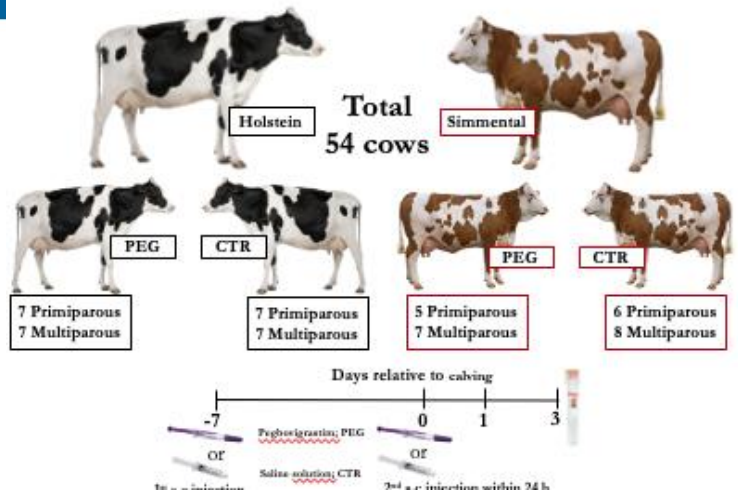


3 DIM: lower haptoglobin & higher Zn levels in IMR cows

Pegbovigrastim treatment alters gene expression profiles of leukocytes after calving

Lopreiato et al. 2020 https://doi.org/10.3390/ani10040621





Pegbovigrastim:

- ✓ elicited migration, adhesion, antimicrobial ability
- ✓ enhanced inflammatory response that in turn mount a better response after the calving event.

Migration and adhesion	PEG effect
CX3CR1 C-X3-C Motif Chemokine Receptor 1	-
ITGB2 Integrin Subunit Beta 2	up
ITGAL Integrin Subunit Alpha L	up
TLN1 Talin 1	up
TLN2 Talin 2	down
SELL Selectin L	up
SELPLG Selectin P Ligand	up
CD44 Hematopoietic Cell E- And L-Selectin Ligand	up
IL8 C-X-C Motif Chemokine Ligand 8	down

Inflammatory response	PEG effect
CASP1 Caspase 1	up
IL1B Interleukin 1 Beta	up
TNFRSF1A TNF Receptor Superfamily Member 1A	up
TNF Tumor Necrosis Factor Alpha	down
IL1R Interleukin 1 Receptor Type 1	up
IL18 Interleukin 18	up
IRAK1 Interleukin 1 Receptor Associated Kinase 1	up
NLRP3 NLR Family Pyrin Domain Containing 3	up
S100A8 S100 Calcium Binding Protein A8	up
RPL13A Ribosomal Protein L13a	down

Killing capacity	PEG effect
IDO1 Indoleamine 2,3-Dioxygenase 1	-
LCN2 Lipocalin 2	up
MMP9 Matrix Metallopeptidase 9	up
MPO Myeloperoxidase	up

Recognition and Infl. Mediation	PEG effect
CD14 Cluster of Differentiation 14	up
CD16 Fc Fragment of IgG Receptor IIIa	up
TLR2 Toll Like Receptor 2	up
TLR4 Toll Like Receptor 24	up
MYD88 Myeloid Differentiation Primary Response 88	up

③ IMMUNONUTRITION

the potential to modulate the activity of the immune system by interventions with specific nutrients (Calder, *BMJ* 2003;327:117–8)

Immuno nutrition

- **Microbiota**
  - Modified gut permeability
  - Improved barrier function
- **Immune system**
  - Improved barrier function
  - Improved immune function
- **Inflammation & its resolution**
  - Attenuation of excessive / chronic inflammation
  - Improved wound healing

Lower risk → Improved clinical outcome

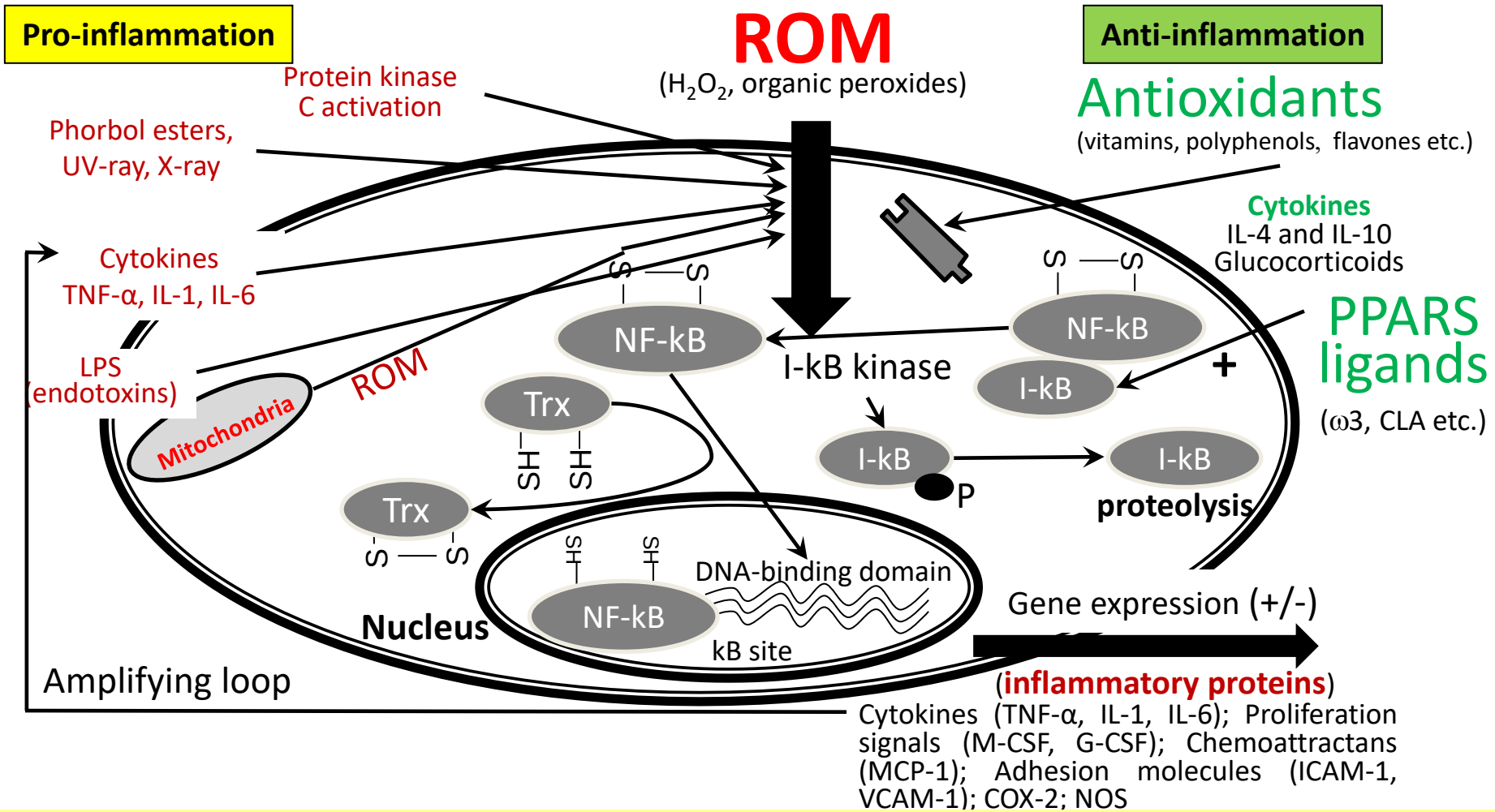
(Calder et al., 2007)

Nutritional factors "pro-resolution":

- **ω_3 fatty acids (& CLA?)**
  - **ligands of PPARs**
  - **competition with ω_6 for lower inflammatory eicosanoid series**
- **some a.a. (met, BCAA)**
- **phyto-products**
- **antioxidants**

***LFI**: tool to assess effects of immunonutrients in TP*

Mechanisms controlling the inflammatory process



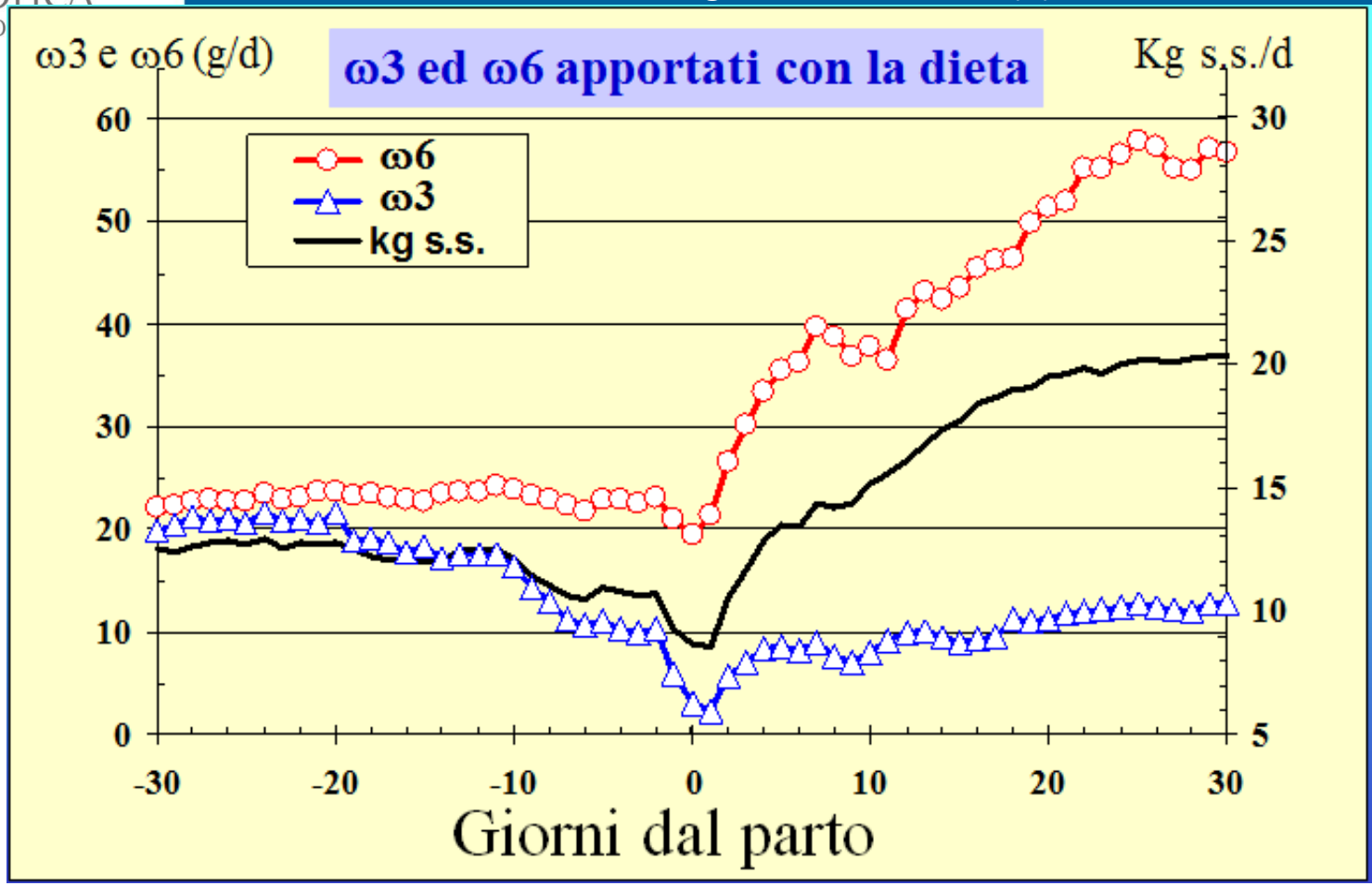
TO AVOID excess of oxidant molecules (but also of antioxidants)

Dairy cows: diet ω3 & ω6

Bertoni et al. 2006. Progress in Nutrition 8(1): 22-27

DIET:

- ✓ **ω3 content starts to decline ~10 d before calving** *(less hay & more grains rich of ω6)*
- ✓ **The reduction of plasma ω3 content precedes the changes of diet**
- ✓ **After calving the diet contribute of ω3 is much less than ω6**



Diet ratio ω6:ω3 (without AG supplement)

dry	1.2 / 2.5 : 1
lactation	5.5 / 10 : 1

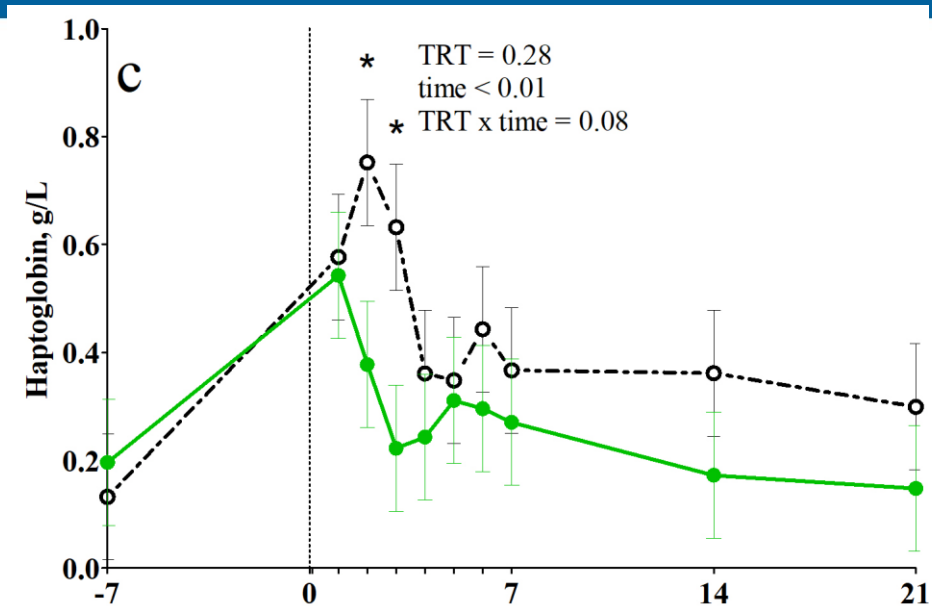
IDEAL: depend to rumen protection and many other factors

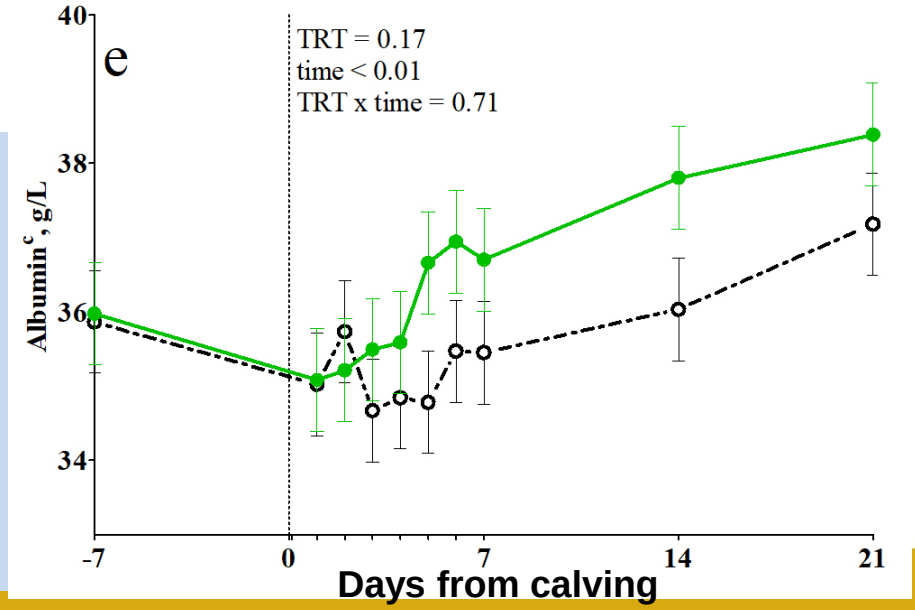
Intrajugular infusion of long chain ω-3 fatty acids at calving

Mezzetti et al. 2022 Frontiers in Veterinary Science *(in press)*

- **TRT (5 cows) i.v. infusion of purified fish oil** *(15 g: 2.3 g EPA + 3.4 g DHA)* **at 12, 36 and 60 h after calving**

- **CTR (5 cows) without infusion**





1st month of lactation:

DMI:

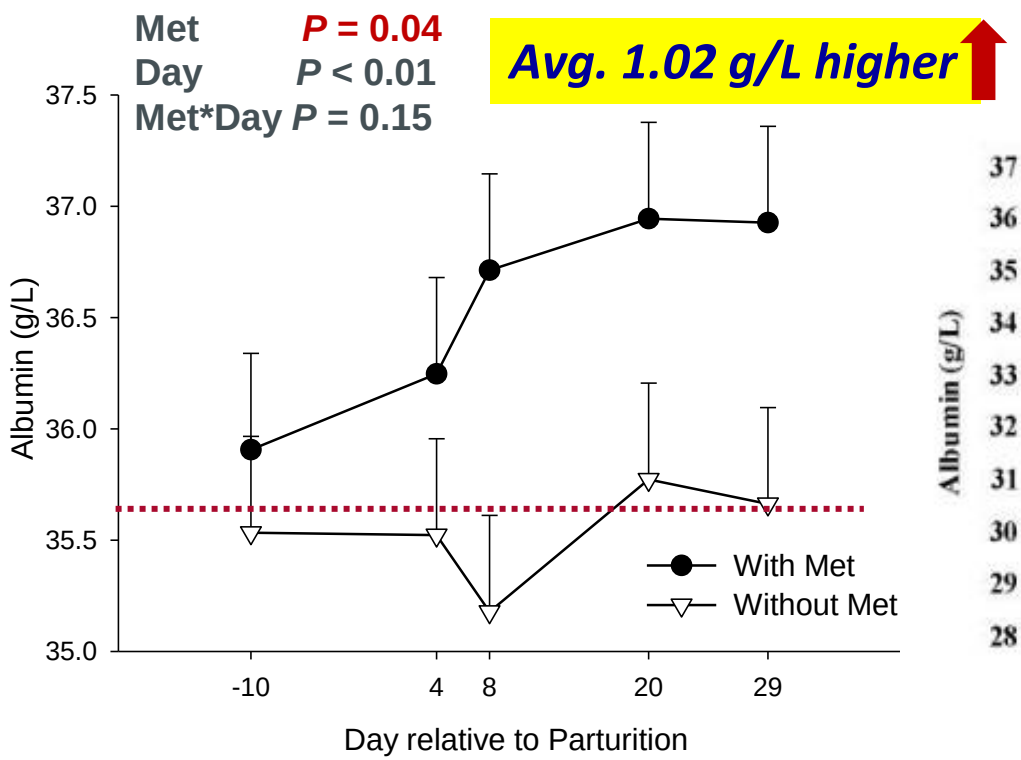
TRT (17.2 kg) vs CTR (16.0 kg) (TRT* time P<0.05)

MY:

TRT (34.4 kg) vs CTR (29.2 kg) (TRT*time P<0.01)

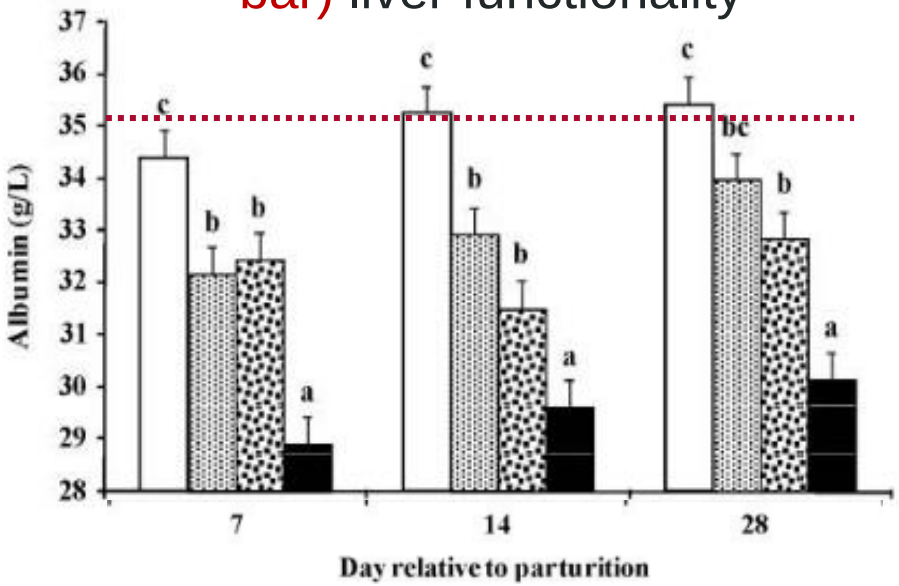
Methionine in TP: better liver function & lower inflammation

Zhou et al. JDS 99:8716–8732 - Zhou et al. JDS 99: 8956-8969



Zhou et al. (2016)

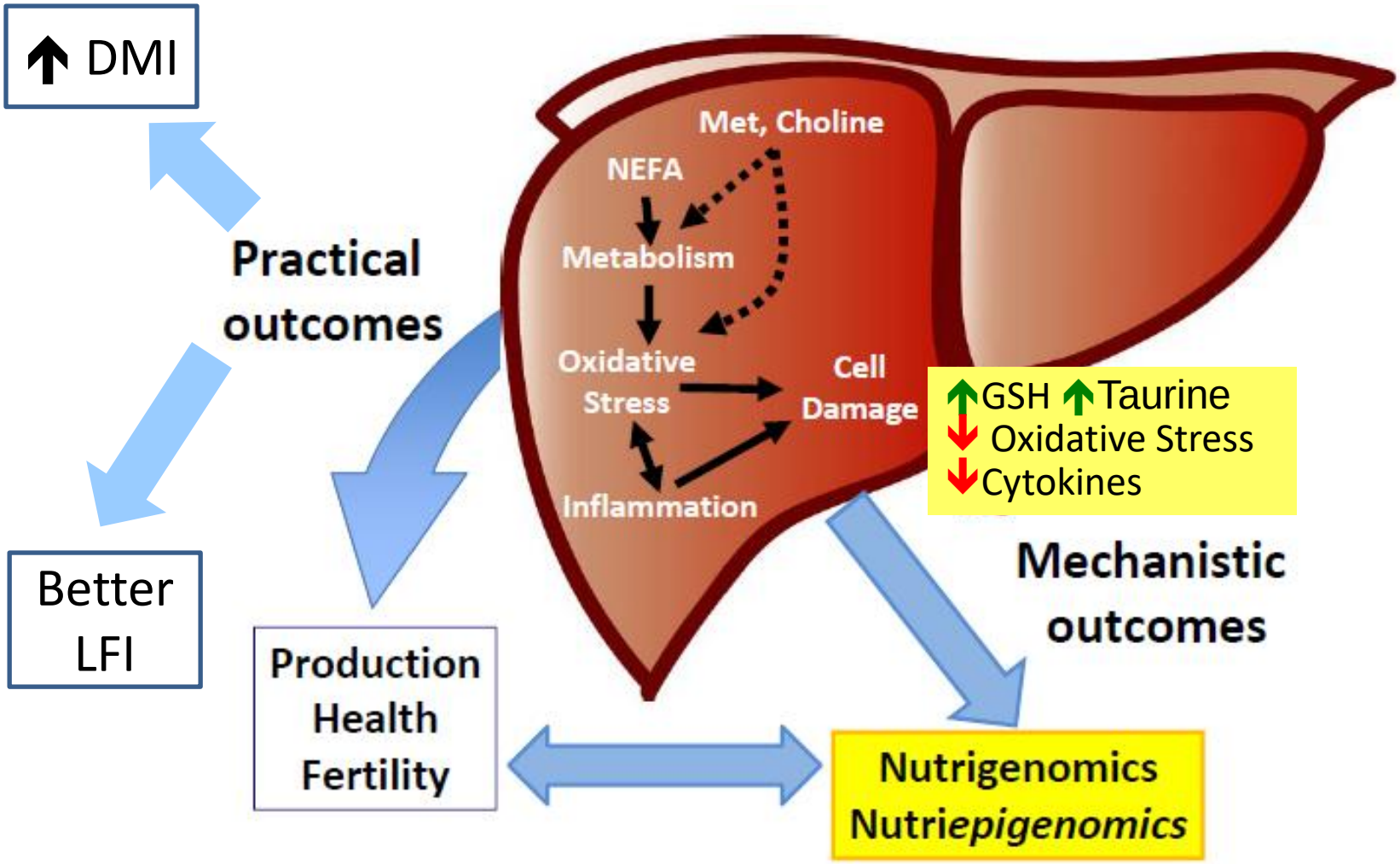
Benchmarks for cows with high (white bar) to low (black bar) liver functionality



Bertoni et. al. (2008 JDS)

Without Met = ~1.85% MP (metabolizable proteins)
With Met = 2.38% MP (dry); 2.15% MP (lact)

Effects of rumen-protected methyl donors in the liver

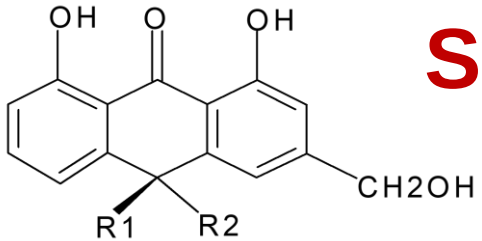


Loor, 2015 (modified)

Aloe arborescens: bioactive molecules

Antrachinons

- aloin A e B
- aloe-emodin
  - effects: cathartic, antineoplastic, antibacterial and / or antiviral



Polysaccharides

- acemannan
  - immunostimulant, anti-inflammatory, healing
  - soothing, anti-diabetic, anti-oxidant
  - antimicrobial, antiviral

Synergic effect?

2/3/6-OAc 2/3/6-OAcα-1,6-Gal

-[ß-1,4-Man]x-[ß-1,4-Glu]x-[ß-1,4-Man]y-[ß-1,4-Man]

Treatment (Trevisi et al., 2013)

150 ml/cow/d homogenate of *Aloe arborescens* (Aloe is Life® - NA, Italy) *from* -14 to +14 days from calving + 100 UI/L α-tocopherol as stabilizer

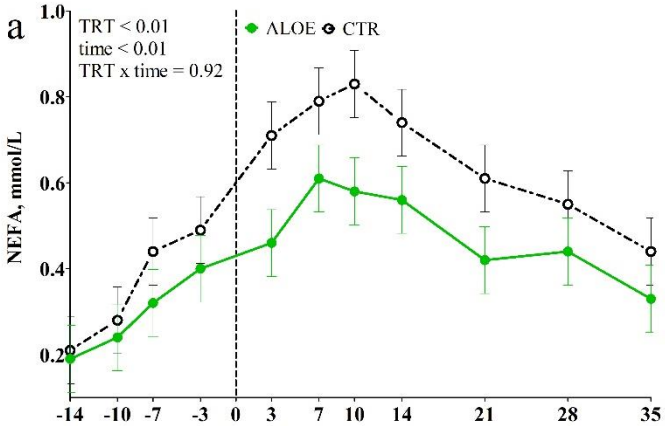
Aloe: lipid metabolism

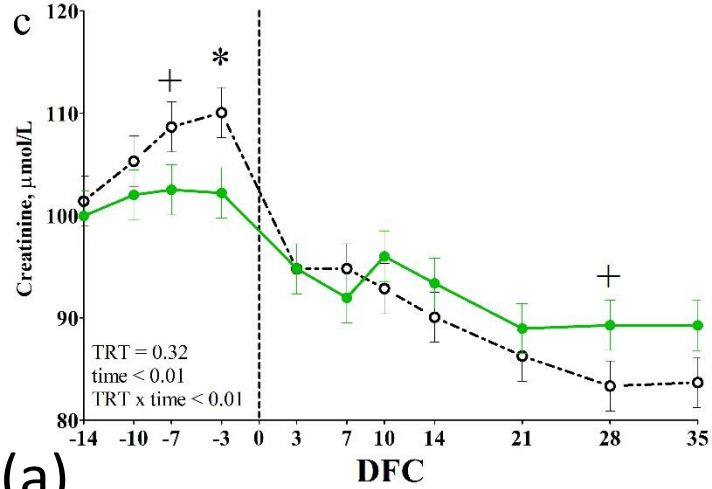
Mezzetti, et al. *Animals* **2020**, *10*, 917.

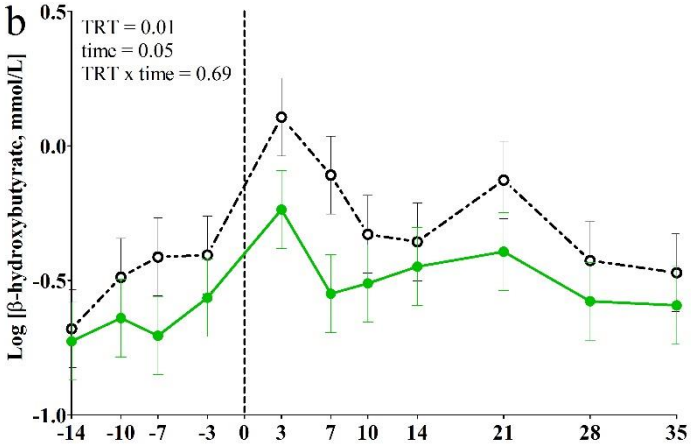
20 multiparous Holstein dairy cows supplemented with:

- 200 g/d water (**CTR**)
- 200 g/d *Aloe arborescens* mill whole plant homogenate (**ALOE**)

Between -14 and 14 days from calving (**DFC**)





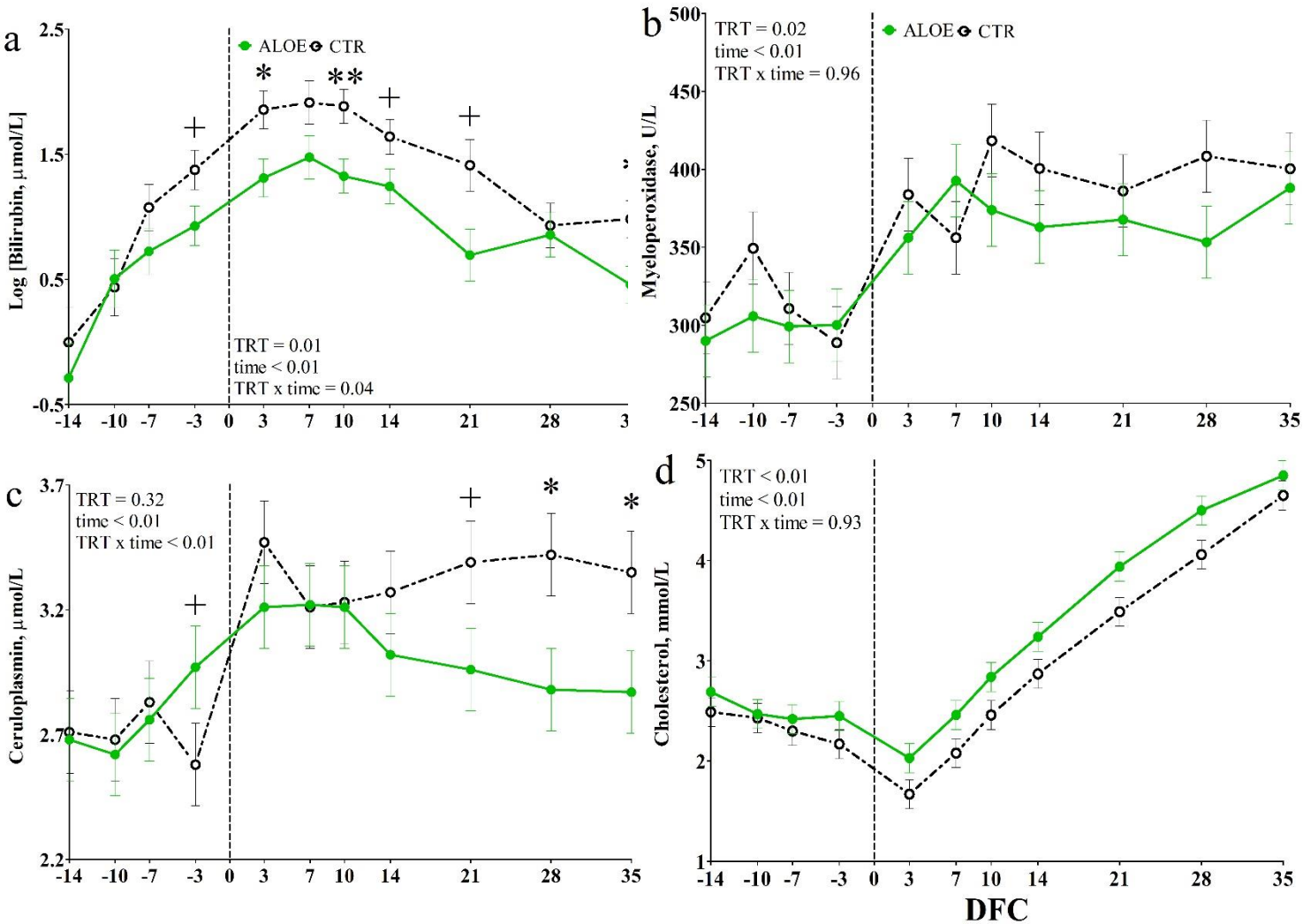


Aloe vs CTR:

- Lower NEFA (a)
- Lower BHB (b)
- Lower creatinine level before calving (c)

Aloe: inflammation

Mezzetti, et al. *Animals* **2020**, *10*, 917.



Aloe vs CTR:

- Lower bilirubin (a)
- Lower MPO (b)
- Lower CuCp after calving (c)
- Higher cholesterol (d)

- higher LFI *(= better recovery after calving)*

IMPLICATIONS

- **Inflammation** is a complex & pivotal process of the body defense *(often metabolic costs and side effects are dangerous)*
- **Critical aspects** are: chronic response, metaflammation, timing of the detection, regulation of resolution
- In dairy cows, **some biomarkers** (e.g. negAPP) help to detect subjects at risk of metabolic diseases

IMPLICATIONS

- Attenuation of inflammation improves performance, health & welfare in TP.
- Thus, **its modulation is necessary:**
  - **Genome: dairy breeds show different infl. responses** under similar challenges *(presence of genetic polymorphisms in some regulatory genes)*
  - **Immunomodulation** *(e.g. by tuning of the immune cells recruitment)*
  - **Immunonutrition is** the most effective strategy *(e.g. balancing ω6 and ω3 ratio, special nutrients)* and require more studies

Many thanks for attention

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Dr. M. Bionaz (Oregon Univ.)
Dr Z. Zhou (Clemson Univ.)
Dr. J. Osorio (South Dakota Univ.)

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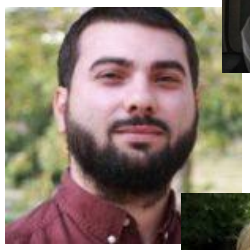
QUESTIONS?

Dr Andrea Minuti

Dr Matteo Mezzetti



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