Micotossine ed endotossine nella bovina da latte: conoscerle e prevenirle. L'aiuto della nutraceutica

Venerdì 29 Gennaio 2016
Centro congressi "Giuseppe Piana" - Facoltà di Agraria

Università Cattolica del Sacro Cuore - Piacenza

Pro Tech

4° CONVEGNO

UNIVERSITÀ CATTOLICA del Sacro Cuore

PROGRAMMA

09:30 Saluti

Prof. Erminio Trevisi *Università Cattolica del Sacro Cuore*

Dr. Paglia Carlo *Pro Tech srl*

09:45 Nuove micotossine nei foraggi: problema emergente

Dr Antonio Gallo *Università Cattolica del Sacro Cuore*

10:15 Controllo delle micotossine negli alimenti e strategie di contenimento

Dott.ssa Giuseppina Avvantaggiato *CNR – ISPRA - Bari*

10:45 Discussione

10:55 Coffee break

11:15 Endotossine: conseguenze sul sistema immunitario e sulle performance della bovina da latte

Prof. Trevisi Erminio e Dott. Andrea Minuti *Università Cattolica del Sacro Cuore*

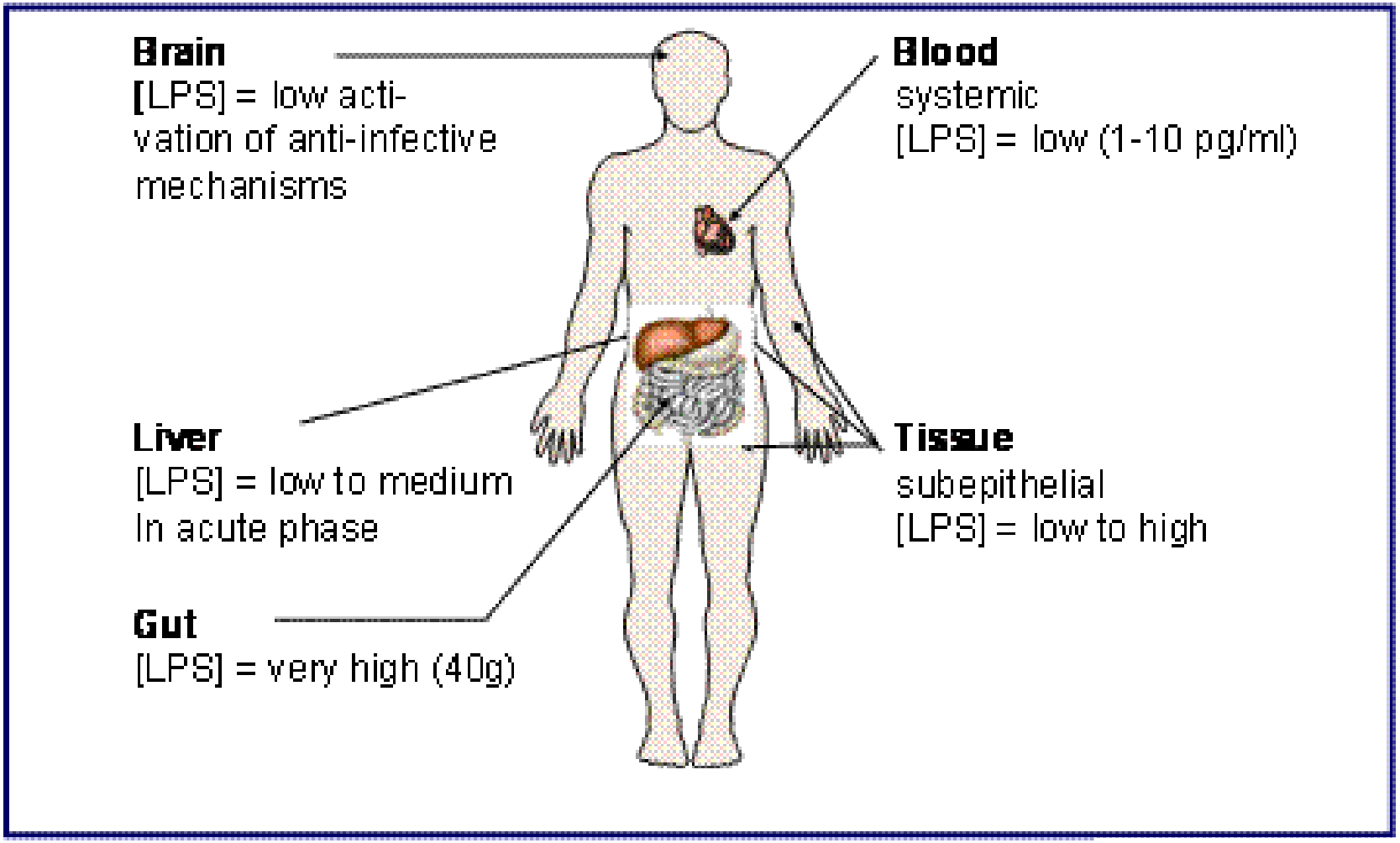
11:45 Capacità complessante di pareti cellulari e derivati di lieviti nei confronti di alcune micotossine e patogeni. Effetti sulla risposta immunitaria delle bovine.

Dr Valentin Nenov *MBA in Veterinary Medicine (Libero Professionista, Ruminant Specialist)*

12:10 Discussione e Considerazioni conclusive

12:30 Brunch

Homo sapiens or Homo Bacteriensis? (10^{13} cellule umane vs 10^{14} batteri)



Perchè occuparci di micotossine ed endotossine?

Ludwig Feuerbach (1804): «*siamo* ciò *che mangiamo*» ….

Ma davvero coincidiamo precisamente con ciò *che* ingeriamo?

Piuttosto siamo il frutto dei fenomeni che evolvono dal rapporto ambiente, alimenti, microbiota, ospite …

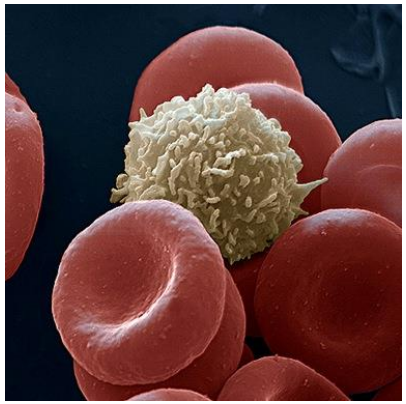
Endotossine: conseguenze sul sistema immunitario e sulle performance della bovina da latte

Erminio Trevisi & Andrea Minuti

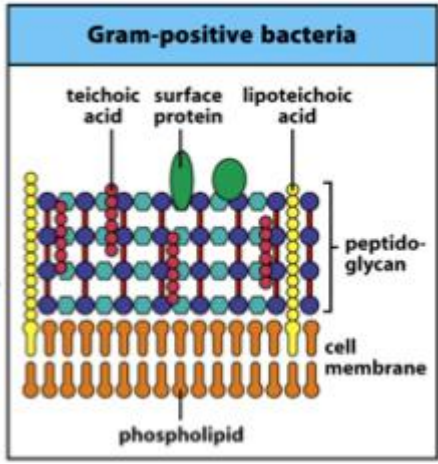
Istituto di Zootecnica
Facoltà di Scienze Agrarie, Alimentari ed Ambientali
Università Cattolica S. Cuore

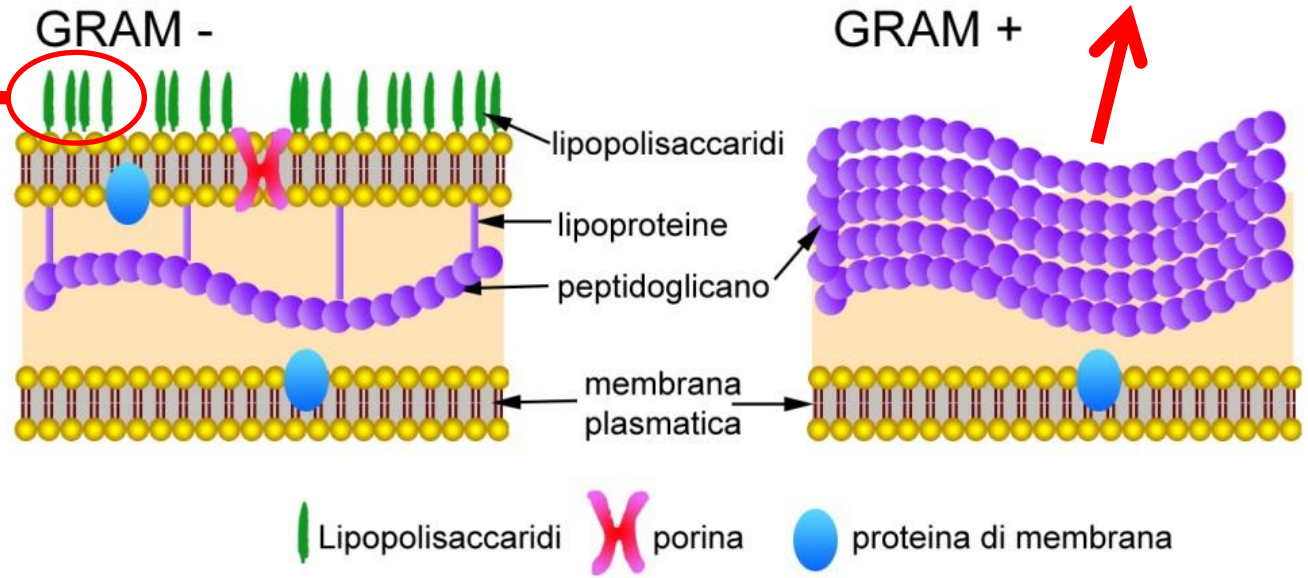
erminio.trevisi@unicatt.it

Lipopolisaccaride (LPS)



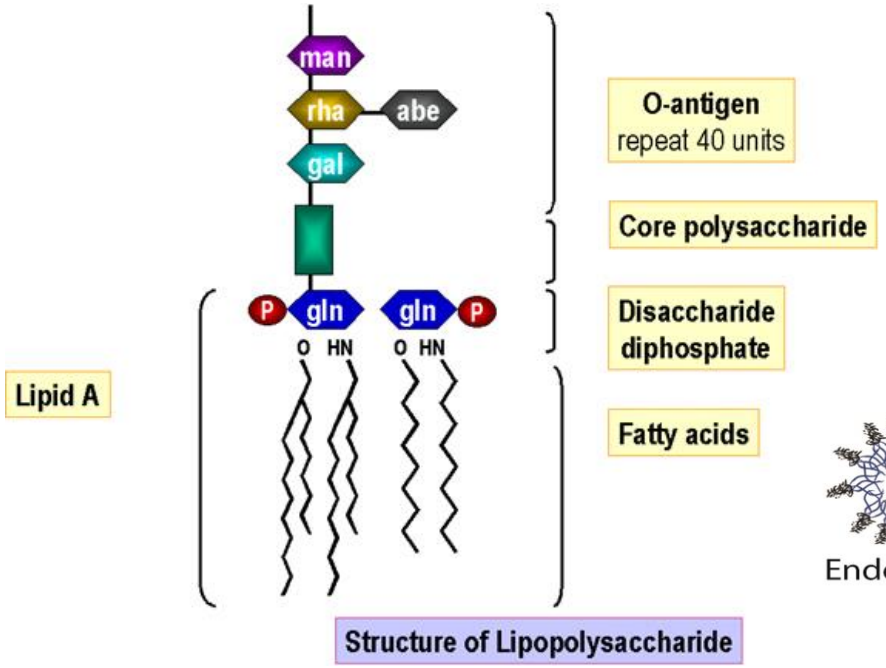
Peptidoglicani +
ac. teicoico o
ac. lipoteicoico

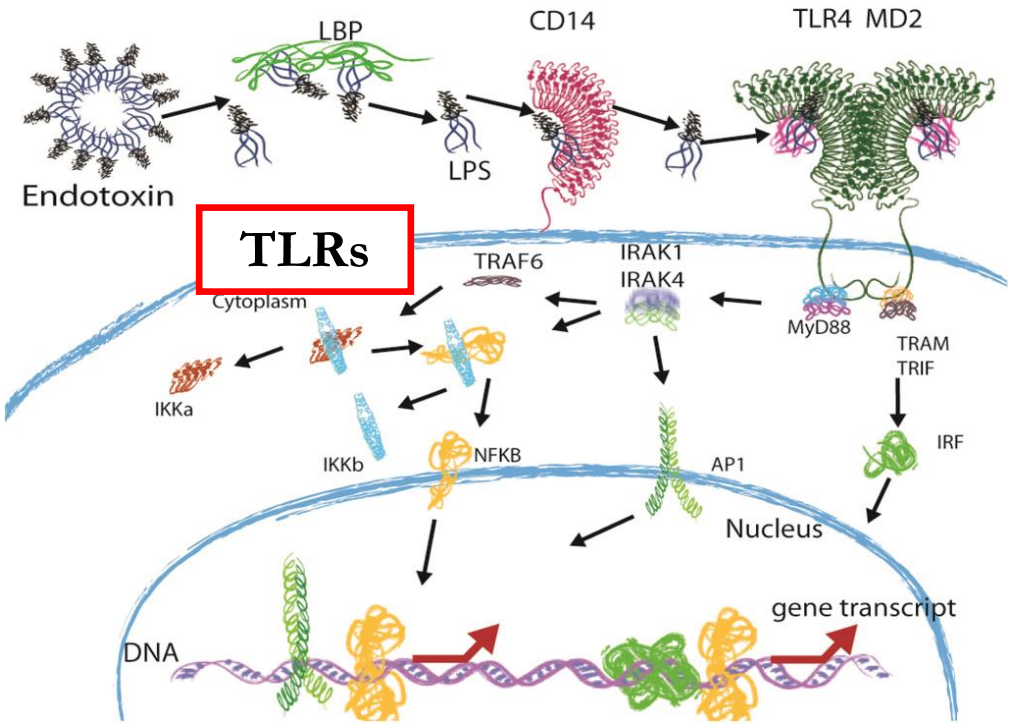




http://en.wikipedia.org/wiki/Lipopolysaccharide

LPS o endotossina





Nijland et al. 2014. *Mar. Drugs* **2014**, *12*(7), 4260-4273

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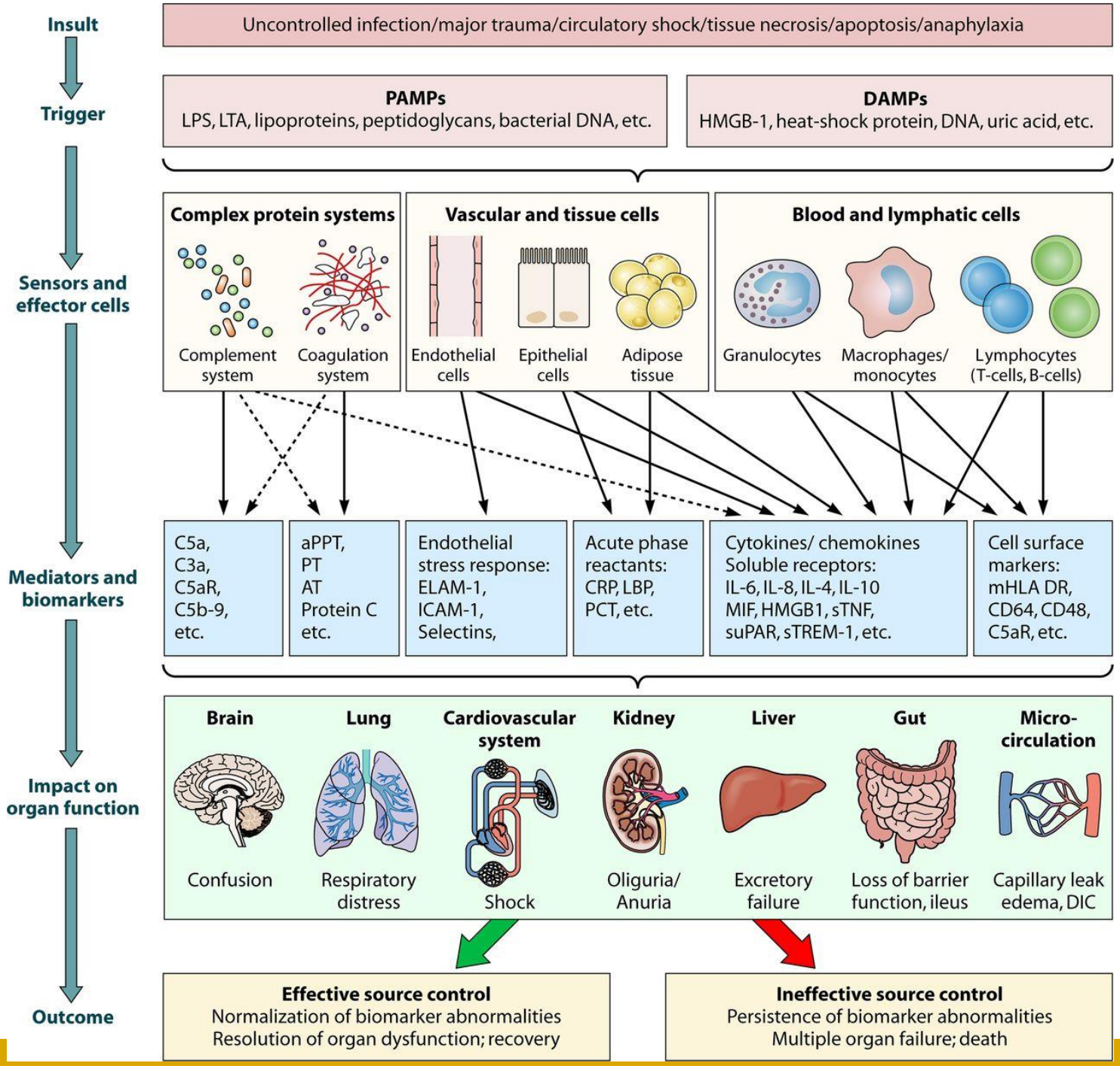
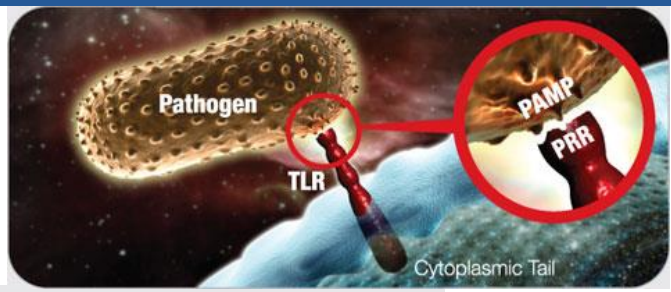
INFIAMMAZIONE



Pattern recognition receptors

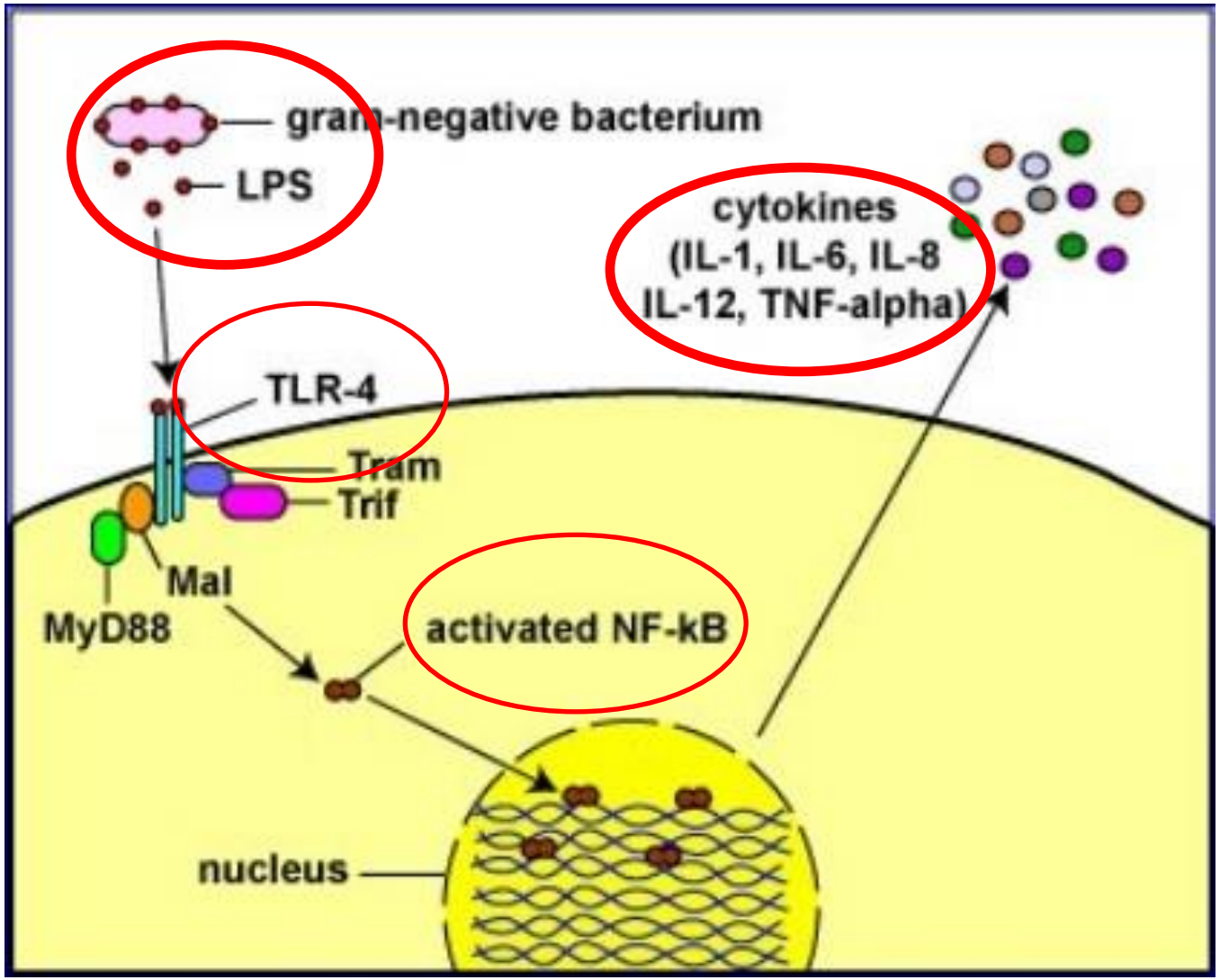


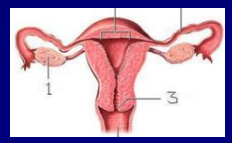
TLRs

NODs & RLRs

S100A8/A9
HMGB1
Profilin
Heparan sulfate
RAGE
LPS
CD14
MD2
TLR11
TLR12
TLR4
Bacterial Cell Wall Compounds
Peptidoglycans
Lipopeptides
Lipoglycans
Flagellin
TLR1
TLR2
TLR6
TLR10
TLR5
C-type Lectin Receptors
N-linked Mannan
Viruses
Mycobacteria
Zymosan
β-Glucan
Fungal Hyphae
N-linked Mannan
SAP130
α-Mannose
Cord Factor
DC-SIGN
Dectin-1
Dectin-2
FcRγ
Mincle
dsDNA
(Viral Infection, Bacteria)
Viral Infection
RNA poly III
Viral RNA
Viral dsRNA
MyD88
TIRAP
MyD88
TIRAP
MyD88
TIRAP
MyD88
TRIF
Translocation
Endosome
ssRNA
CpG DNA ODNs
dsRNA
TLR4
TLR7/8
TLR9
TLR3
CD14
Ras
MAPK p38
TRAM
TRIF
MyD88
TRIF
TRADD
RIP1
MAPK p38 JNK
Raf-1
MAPK
NIK
Syk
Syk
PKCδ
CARD9
MALT1
Bcl-10
β-Catenin
ATP + GTP
cGAS
cGAMP
ER
STING
DNA Sensors
RNA poly III
DAI
MRE11
LRRFIP1
DDX41
IFI16
AIM2
DHX9/36
Cytosolic DNA Sensors
RNA Sensors
RIG-I
MDA5
LGP2
LSm14A
PKR
Bcl-10
CARD9
MAVS
Mitochondria
PAMPs / DAMPs
Inflammasomes
NLRP1, NLRP3, NLRP6, NLRC4, RIG-I, AIM2
Caspase-1 (Caspase-5)
Asc
Caspase-1 Activation
MDP
iE-DAP
Nod2
Nod1
CARD9
RIP2
P
eIF-2α
Protein Synthesis
AP1
NF-κB
IRF3
IRF7
NF-κB
IRF3
IRF7
Cytoplasm
Pro-inflammatory Cytokines & Chemokines
Type I IFNs
Transcription
Nucleus
pro-IL-1β
IL-1β (secreted)
pro-IL-18
IL-18 (secreted)
Inflammatory Response
Inflammation, Immune Regulation, Autophagy, Survival, Proliferation, Apoptosis, Necrosis/Necroptosis

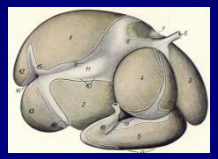
Meccanismo dell'LPS





UTERO

Anomalie digestive { rumine, intestino



Infezioni (patogeni)

GH. MAMMARIA

ENDOTOSSINE

macrofagi

INFIAMMAZIONE

mastociti

istamina

Shock anafilattico

Citochine *Proinfiammatorie (PIC)*

SNC: anoressia

RUMINE: minore motilità

minore assorbimento sostanze nutritive

Ipotalamo: febbre

T. adiposo:
↑ Lipolisi
Pancreas:
↑ glucagone

IPOFISI: CRH

CORTECCIA SURRENALE: glucocorticoidi

antiinfiammatorio

Es. ↓ PIC, PG, metallotioneina

FEGATO
↑Zn metallotioneina
↑ Ferritina

aumenta proteine "fase acuta"

↑ aptoglobina
↑ ceruloplasmina (Cu)
↓ zinco
↓ ferro

sangue

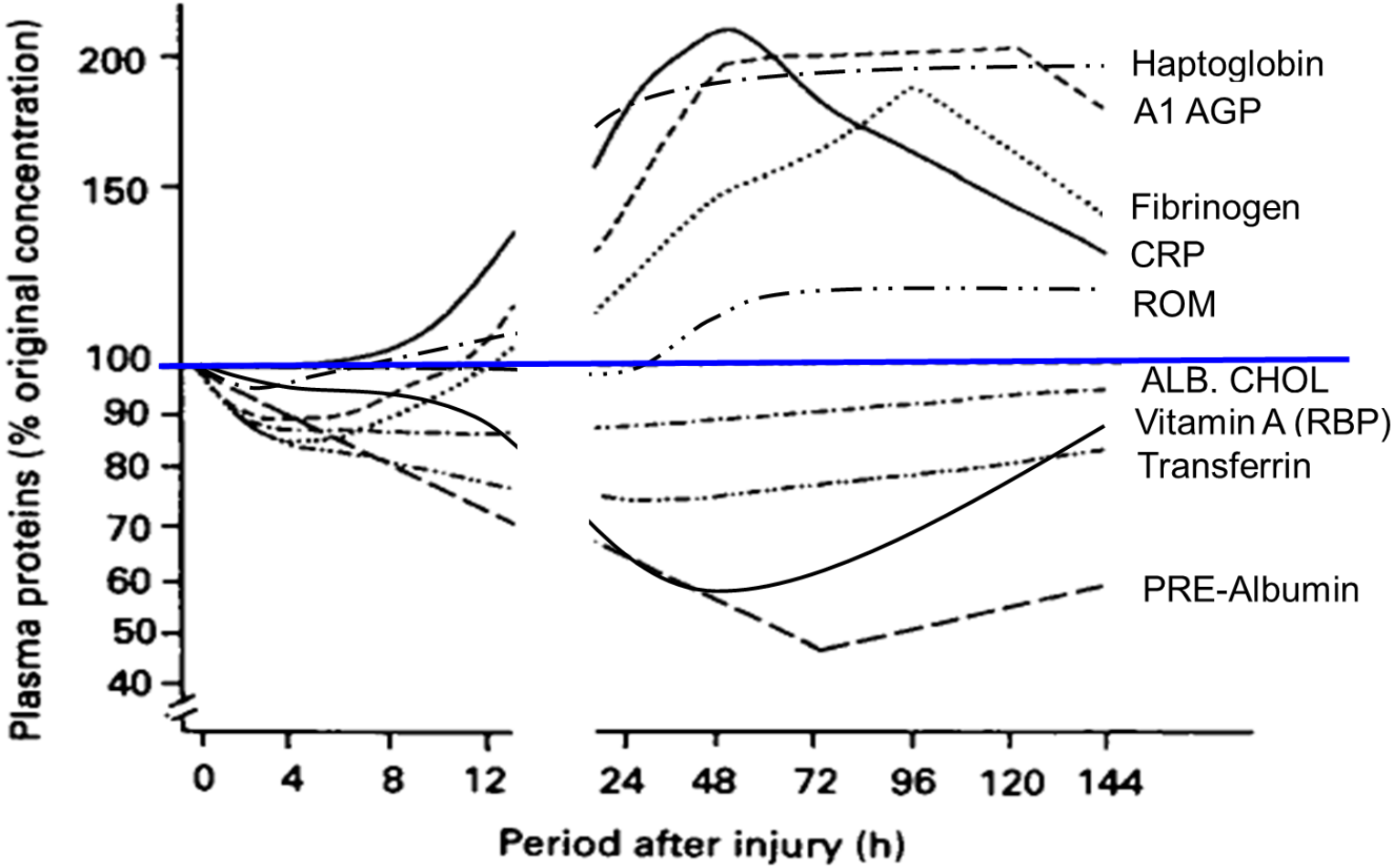
captazione

riduce sintesi consuete

sangue

↓ albumine
↓ lipoproteine
↓ "carriers" di vitamine ed ormoni

Acute Phase Proteins (+APP & -APP)



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 our data).

L'attivazione dell'infiammazione e risposta di fase acuta (APR)

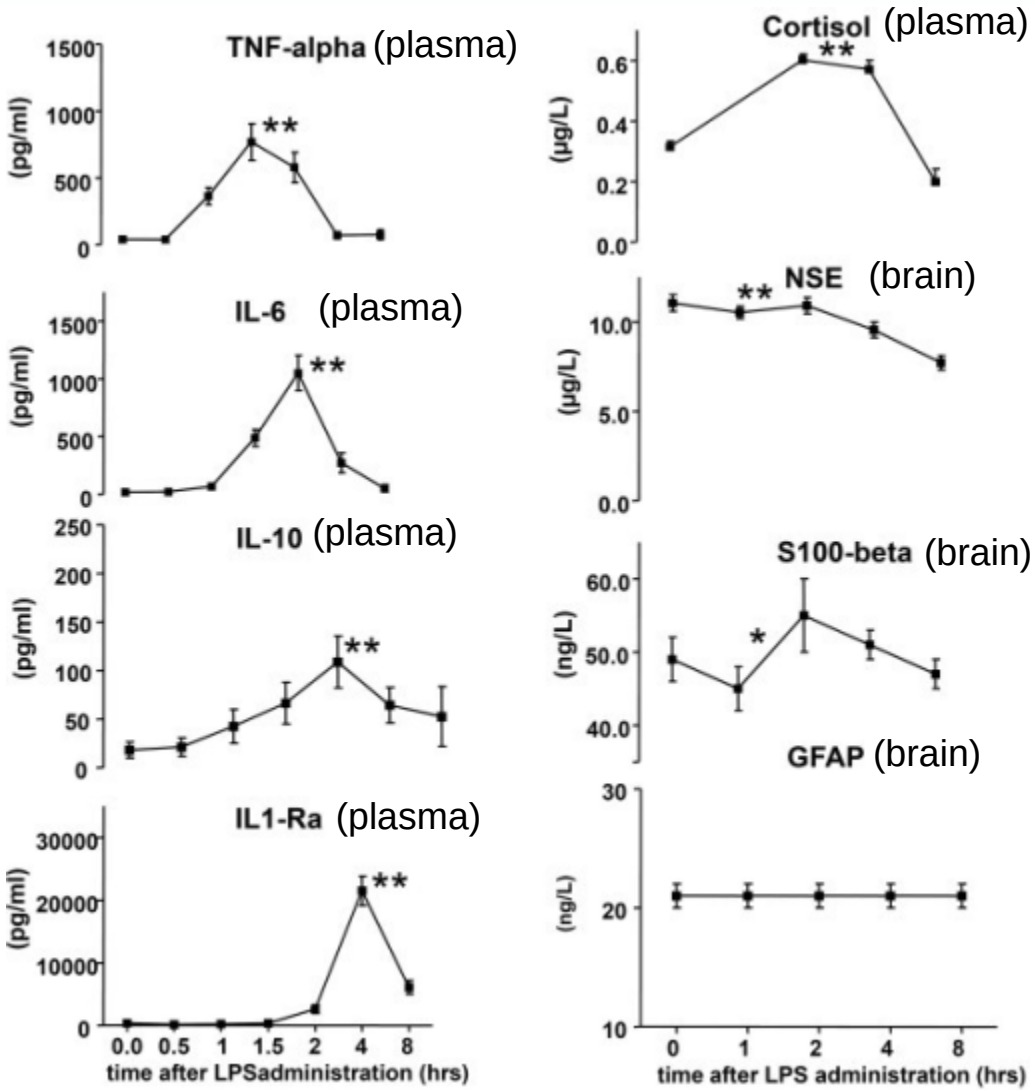
LPS model (humans)

LPS (2 ng/kg) induced changes in cytokine plasma concentrations, cortisol and brain specific proteins. Time 0 reflects baseline concentrations.

Administration of LPS increased: TNF-α, IL-6, IL-10, IL-1Ra, cortisol concentrations (P<0.001) in plasma

[van den Boogaard M et al., 2010; Crit Care 14(3): R81]

NSE = neuron specific enolase
GFAP = glial fibrillary acidic protein;
S100β = S100 Ca-Binding Protein B



Attivazione infiammazione e APR (bovina da latte)

Fig. 12 – Variazioni ematiche dello zinco in bovine trattate con endotossina da E. Coli (3 iniezioni in vena ogni 3 ore od infusione per 24 ore) o con istamina (24 ore infusione)

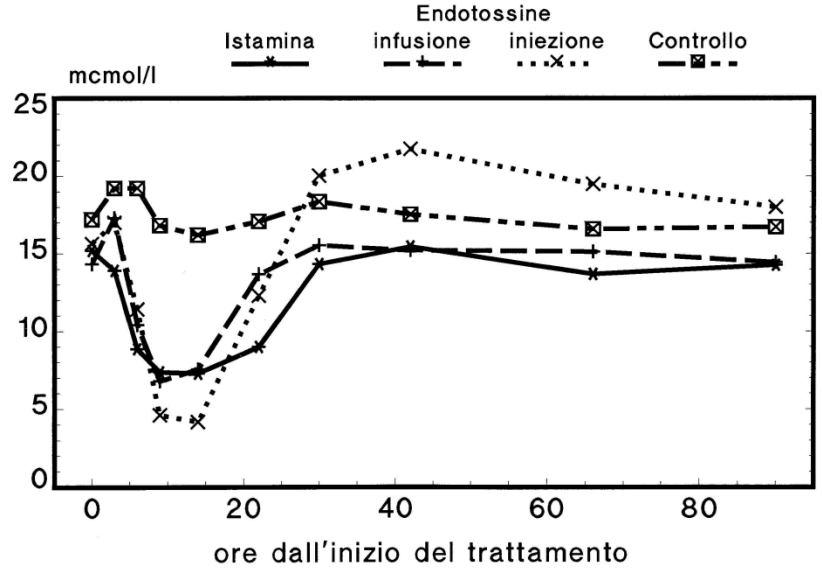
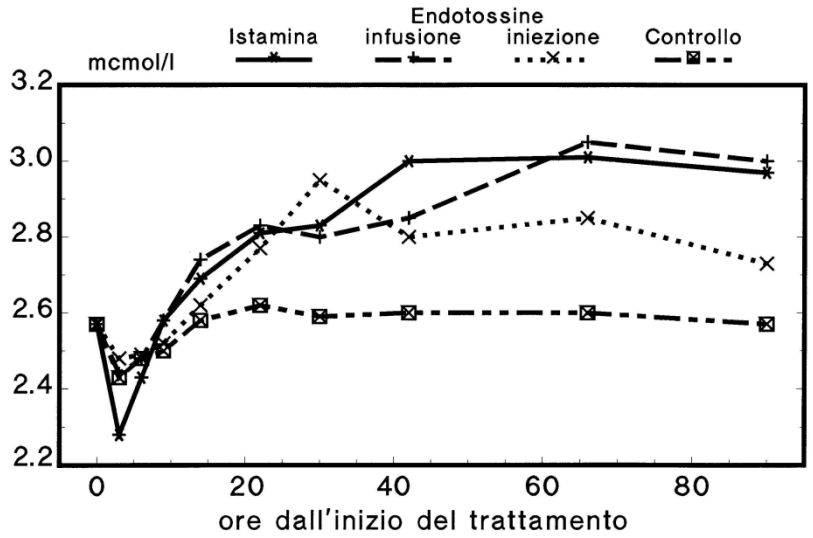


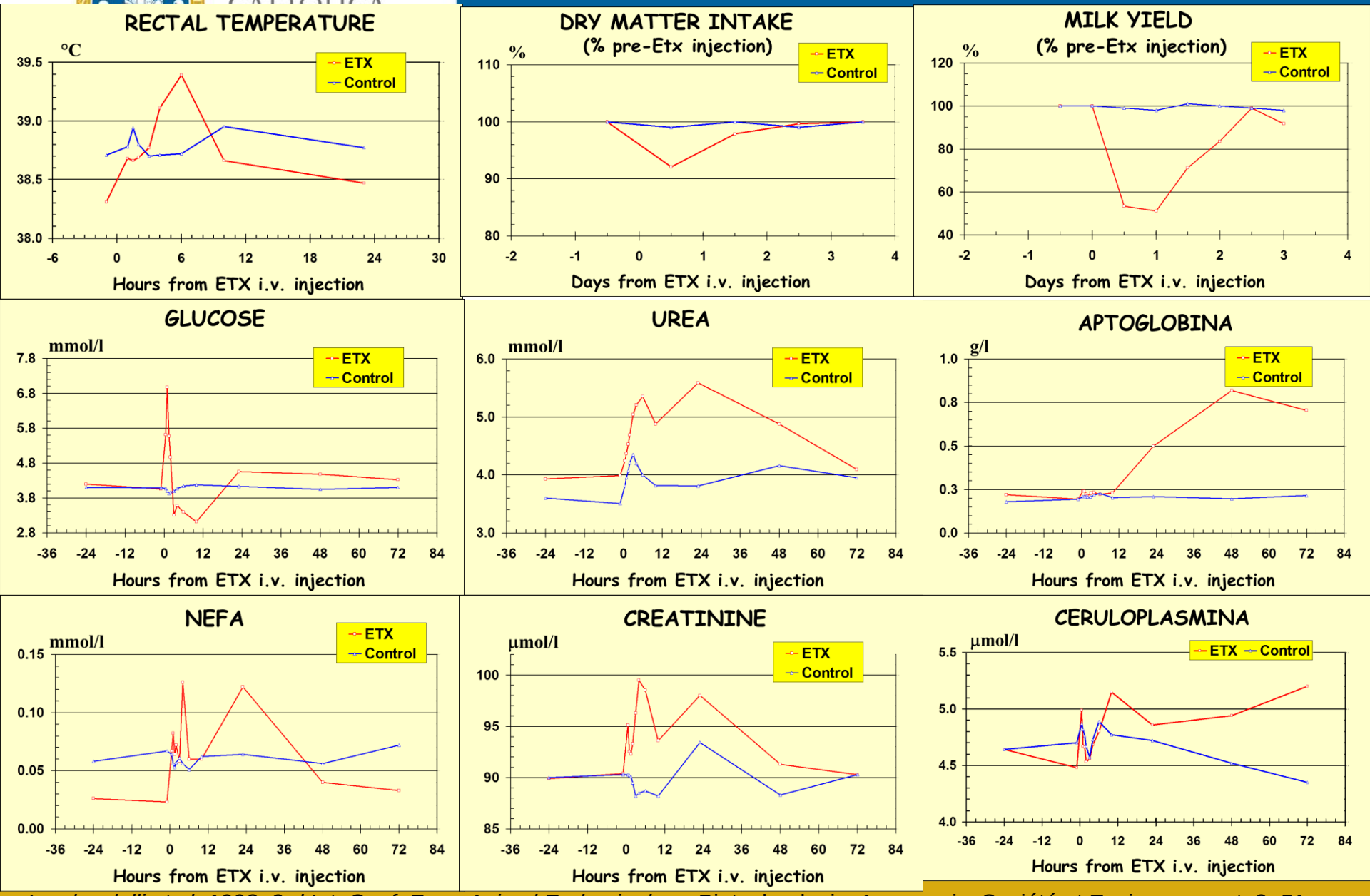
Fig. 13 – Variazioni ematiche della ceruloplasminma in bovine trattate con endotossina da E. Coli (3 iniezioni in vena ogni 3 ore od infusione per 24 ore) o con istamina (24 ore infusione)



Effects of LPS & histamine (not other amines) i.v. infused in dairy cows on blood indices (Bertoni et al., 1989)

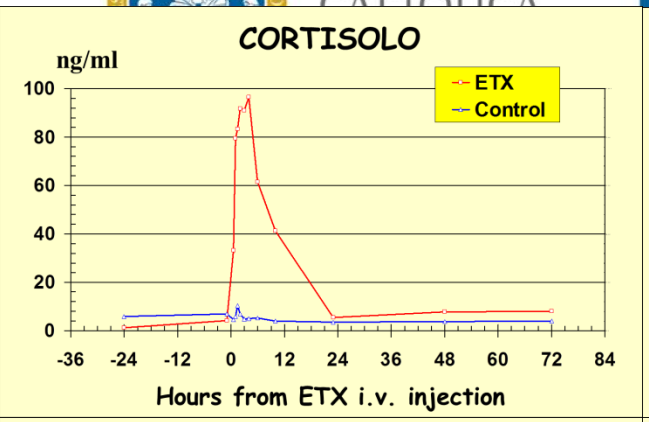
	LPS	Histamine
P_i	↓	↓
Ca	↓	↓
Na	↑	↑
Zn	↓	↓ (quick)
Cucp (+APP)	↑	↑ (slow)
T_3	↓	↓
Insulin	↑	↑
Cortisol	↑	↑
Albumins (-APP)	↓	↓
Retinol BP (-APP)	↓	↓

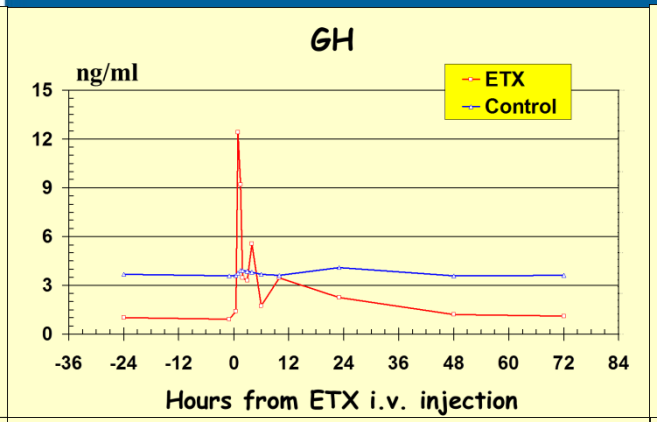
Attivazione infiammazione e APR (bovina da latte)

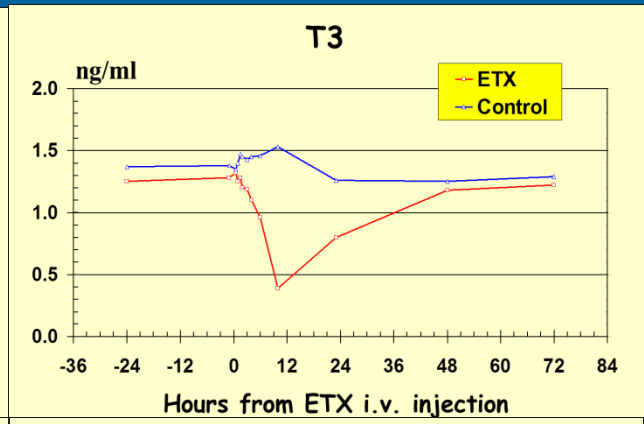


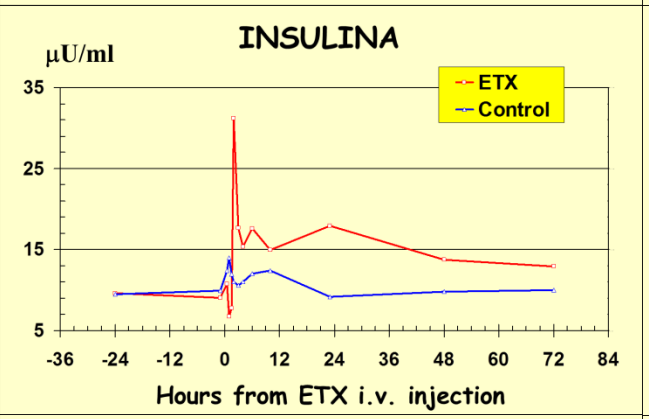
Lombardelli et al. 1998. 3rd Int. Conf. Farm Animal Endocrinology. Biotechnologie, Agronomie, Société et Environnement, 2: 51.

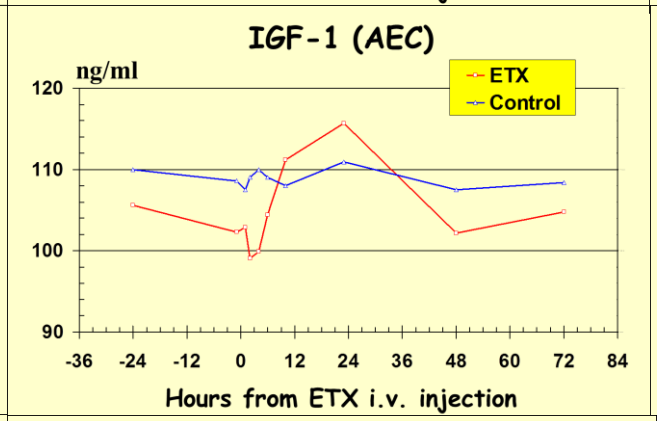
Attivazione infiammazione e APR (bovina da latte)

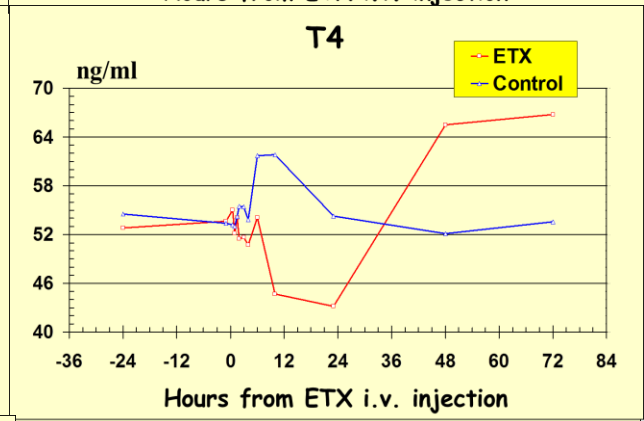


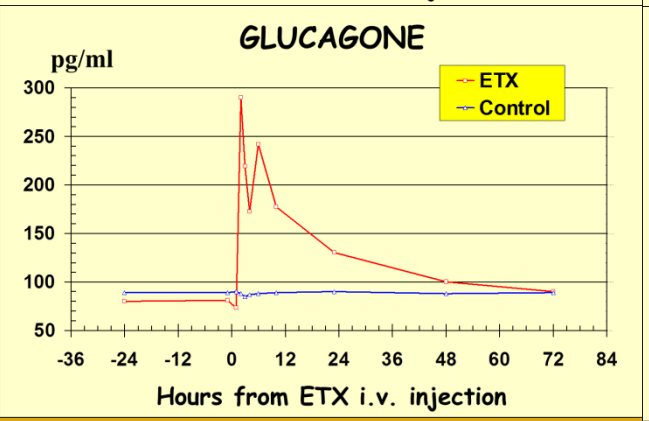


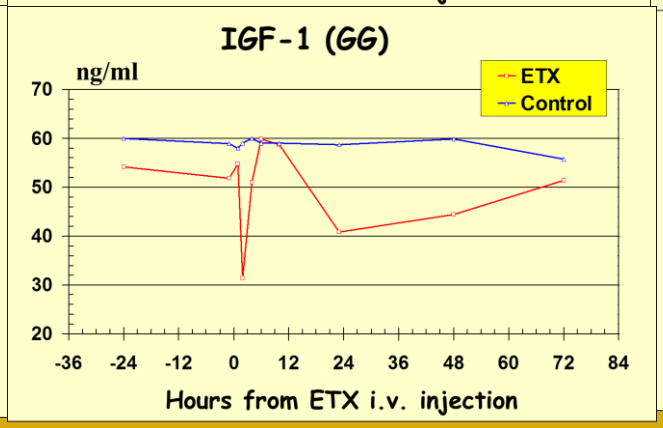


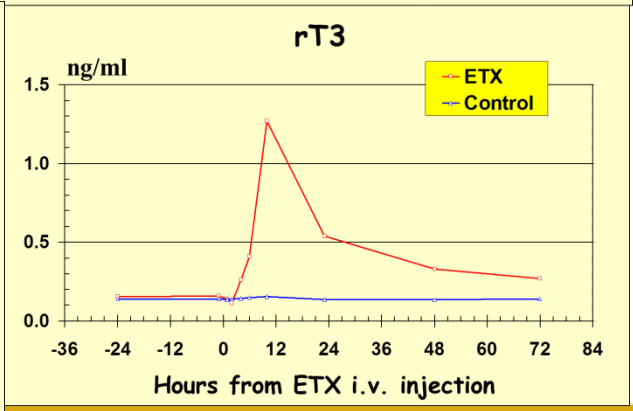












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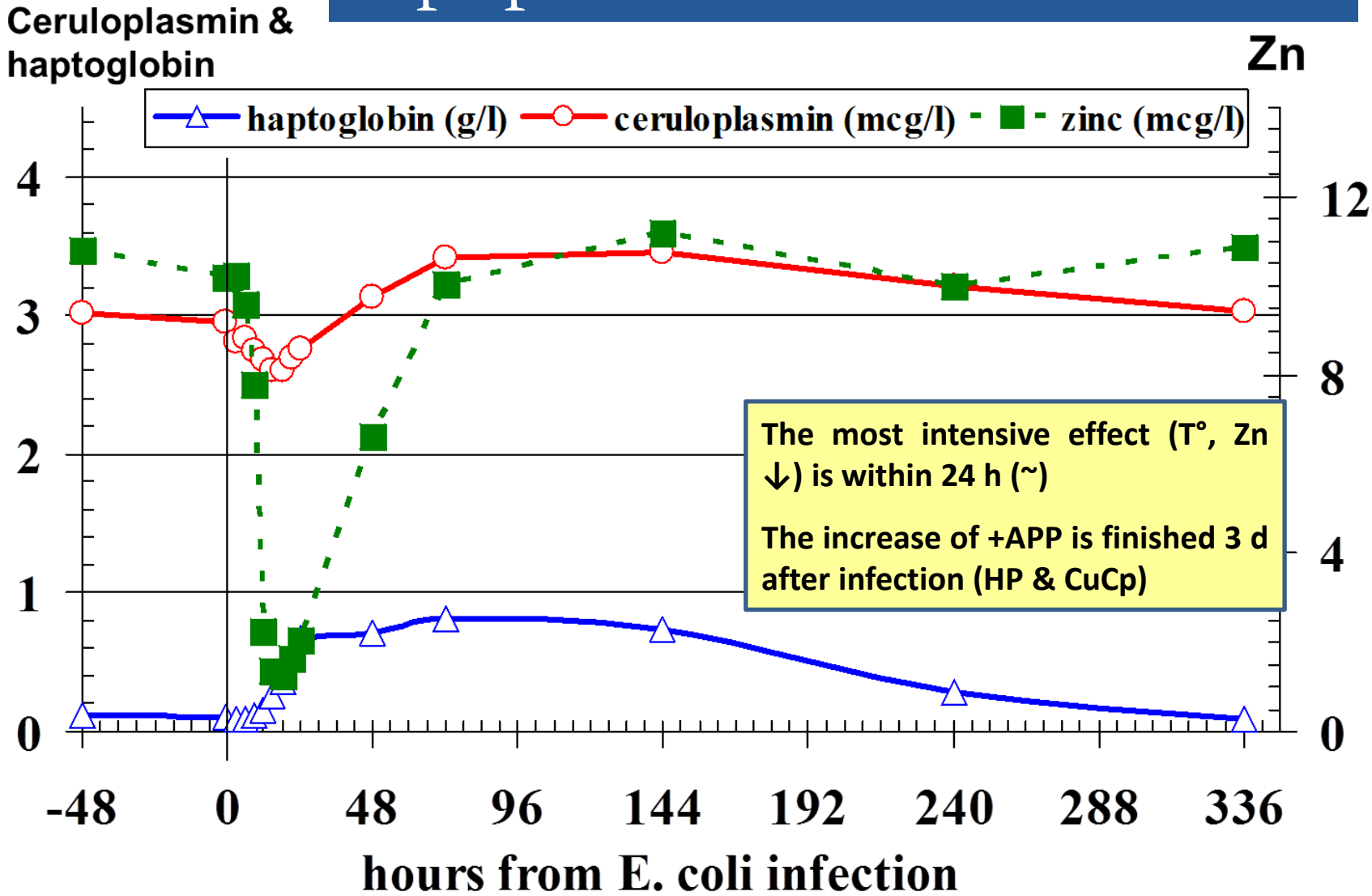
Lipopolisaccaridi e mammella



Data from our Institute (Gubbiotti, 2008) in cooperation with C. Burvenich (Ghent-Belgium) in dairy cows artificially infected with E. coli

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Lipopolisaccaridi e mammella

Physiological Reports

Open Access

Physiological Reports ISSN 2051-817X

ORIGINAL RESEARCH

Acute mammary and liver transcriptome responses after an intramammary *Escherichia coli* lipopolysaccharide challenge in postpartal dairy cows

Andrea Minuti[1], Zheng Zhou[2], Daniel E. Graugnard[2], Sandra L. Rodriguez-Zas[2], Alejandro R. Palladino[3], Felipe C. Cardoso[2], Erminio Trevisi[1] & Juan J. Loor[2]

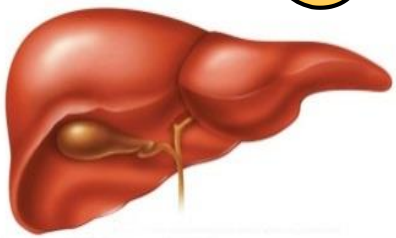
1 Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy
2 Department of Animal Sciences, Division of Nutritional Sciences, University of Illinois, Urbana, Illinois
3 Facultad de Agronomia, Universidad de Buenos Aires, Buenos Aires, Argentina

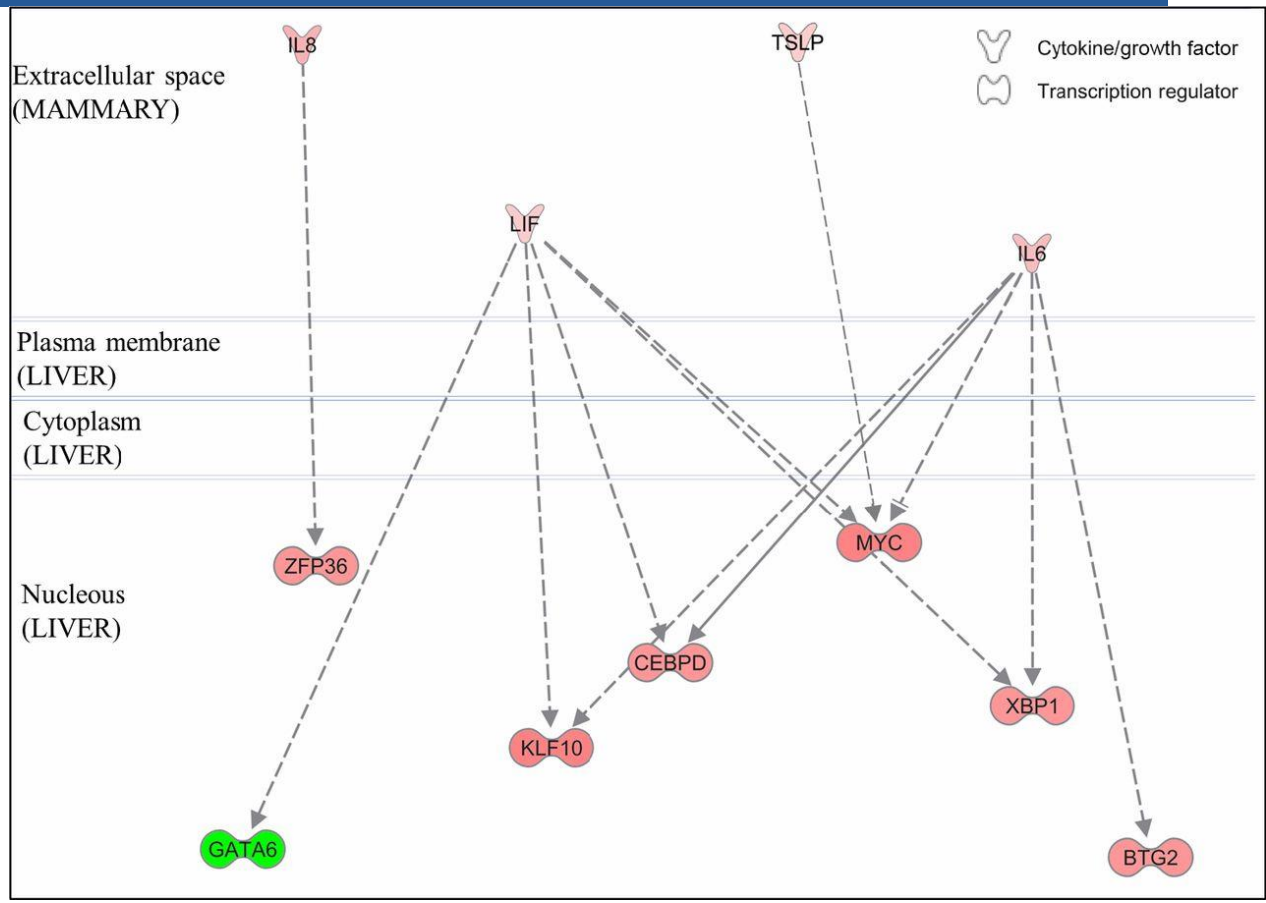
Minuti et al., *Physiological Reports* 2015, 91:5646-5653.

LPS e mammella

LPS = 200 µg, strain 0111:B4

~2.5 h





- ✓ LPS in mammella induce una rapida e severa risposta epatica
- ✓ Mammella: attivazione recettori che richiamano leucociti
- ✓ Sangue: LPS? Rilascio vari mediatori
- ✓ Fegato: modificata funzionalità (APR e modificato metabolismo lipidico)

Minuti et al., *Physiological Reports* 2015, 91:5646-5653.

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LPS e utero

Retained Placenta cows had **higher LPS levels** in uterine lochia (average of 2.24 x 10^4 Endotoxin Units (EU)/mL) vs dystocia & healthy postpartum cows (0.10 & 0.26 EU/mL)
LPS were not observed in plasma: (i) not absorbed into the blood, (ii) efficiently detoxified by IgG anti-LPS or other detoxification mechanisms.

(Dohmen et al., 2000, Theriogenology 54(7):1019-32)

Clinical case of **bovine metritis** (2-year-old lactating Holstein),

- fever >39.5°C,
- fetid, watery, red-brown uterine discharge from the vagina
- LPS in plasma & uterine fluid were 0.94 and 6.34 endotoxin EU/mL
- **1** of 7 **follicles** showed an **extremely high level of LPS** (12.40 EU/ml) compared to the other (0.62–0.97 EU/m*l*) (Magata et al., 2015. J. Vet. Med. Sci. 77(1):81-84)

Cow endometritis: **peak of plasma LPS = 0.08-9.14 EU/ml, between 1 & 12 DIM**

(i) LPS & PGE2 (uterine fluid) and of plasma PGFM: related to degree of endometritis
(ii) absorption of LPS from the uterus to the bloodstream: **in heavy endometritis**
(iii) relationship between uterine infection, LPS & resumption of pp ovarian activity

(Mateus et al., 2003, Anim Reprod Sci. 76:143-54)

LPS e utero

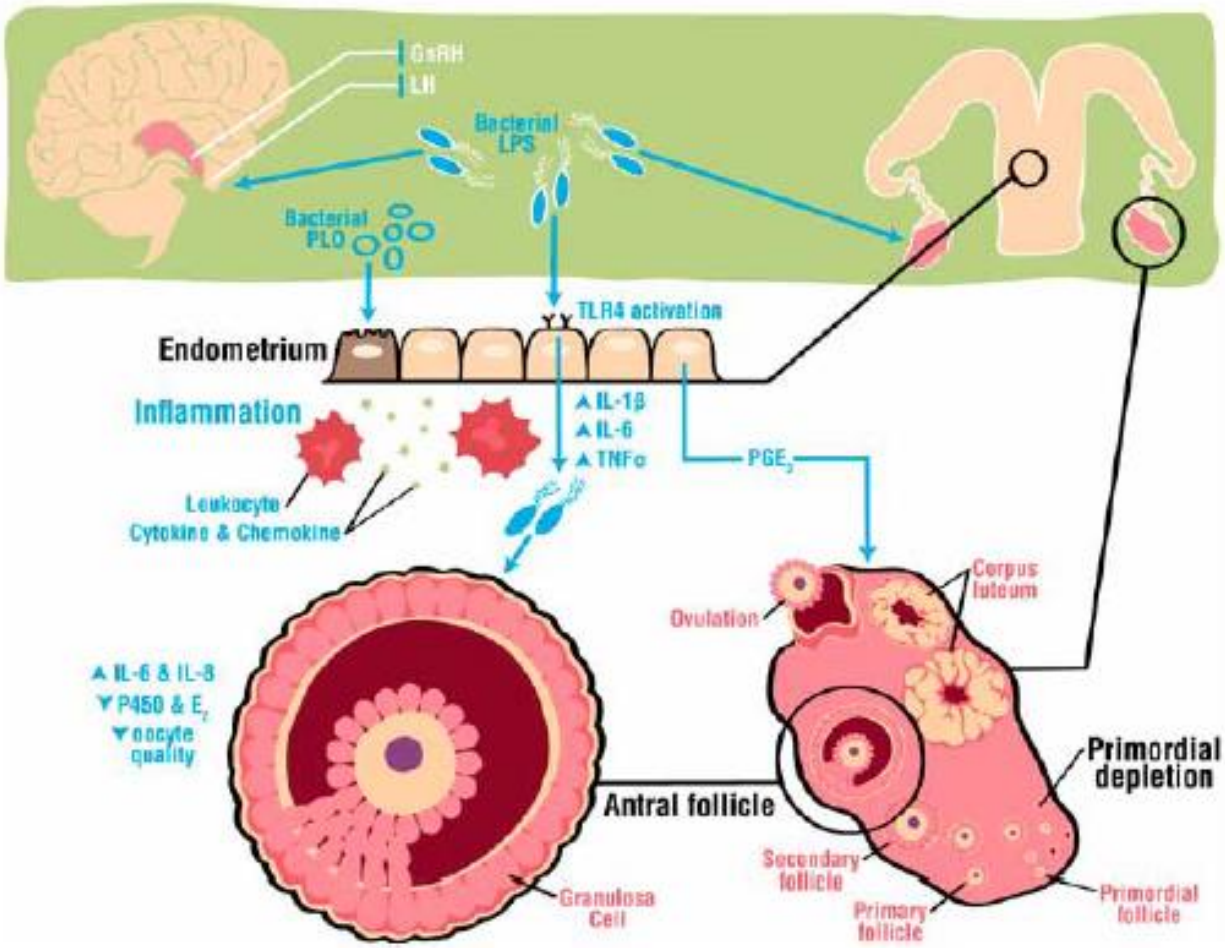


Illustration by Stacey Jones (University of Florida)

Effects of uterine bacterial infection on neuroendocrine signaling, uterine health, and ovarian function:

Brain: ↓ GnRH and LH

Endometrium: bacterial pyolysin disrupts endometrial cells by osmotic lysis. LPS initiate an inflammatory response via TLR-4 activation (↑ cytokine, chemokine, & PGE2 production).

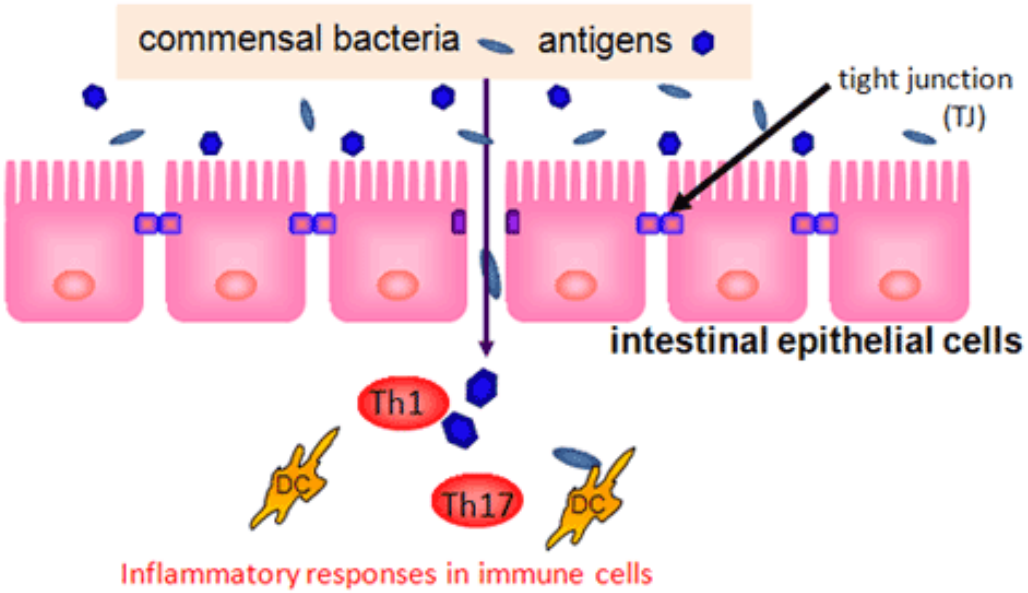
Ovary: primordial follicle reserve is depleted, follicle growth is retarded, & luteal phase is prolonged.

Ovarian granulosa cells respond to bacterial LPS in a TLR4-dependent manner, increasing inflammatory mediators, reducing aromatase and estradiol, and reducing oocyte competence.

Bromfield et al., *J. Anim. Sci. 2015.93:2021–2033*

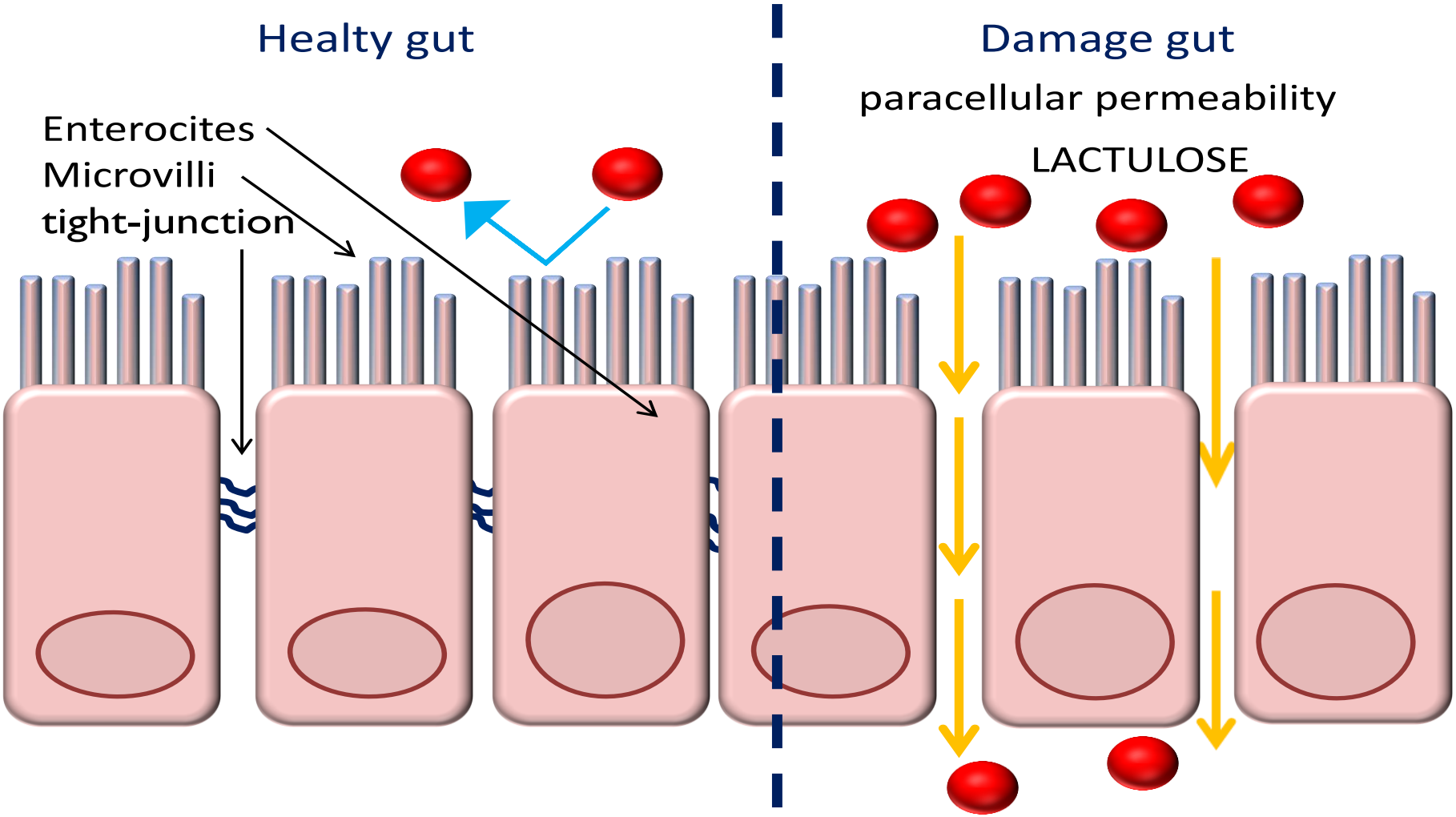
Alterata permeabilità GIT

Una *minima permeabilità del GIT c'è sempre*, ma il sistema immunitario è normalmente in grado di evitare che gli agenti patogeni causino danni.



Un aumento della permeabilità può portare a pericolose reazioni infiammatorie locali e sistemiche.

Misurazione permeabilità GIT



Malattia di Crohn & colite ulcerosa

Analisi lattulosio: livelli più elevati in urine, ma buona relazione con livelli ematici *(sangue «più facile in animali»)*

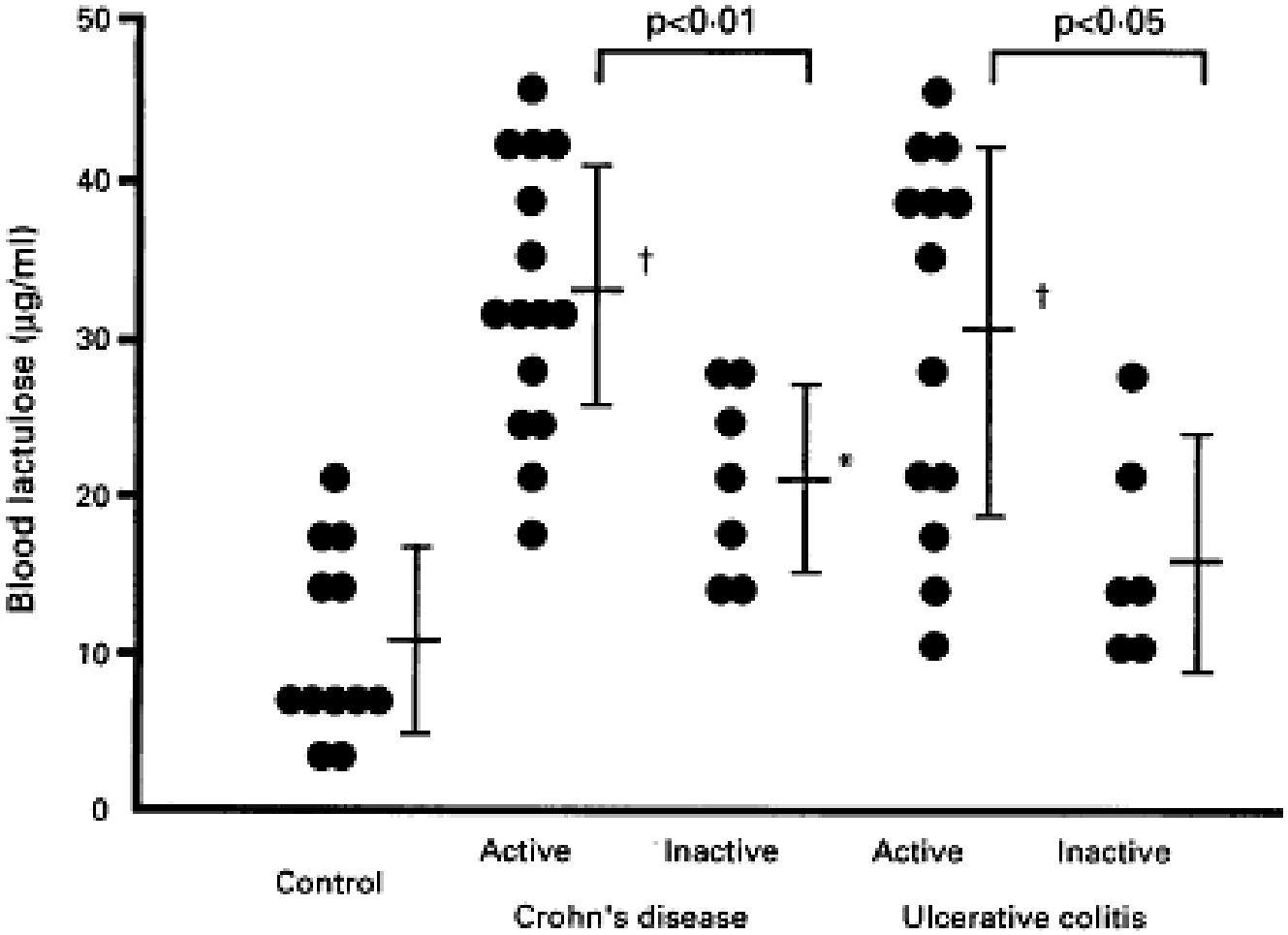


*Figure 3: Comparison of the maximum blood lactulose concentrations (mean (SD)) in patients with Crohn's disease and ulcerative colitis. Each point represents the maximum blood lactulose concentration in each tolerance test. *p<0·01 and † p<0·001 v controls (Mann-Whitney U test).*

Malattia di Crohn & colite ulcerosa

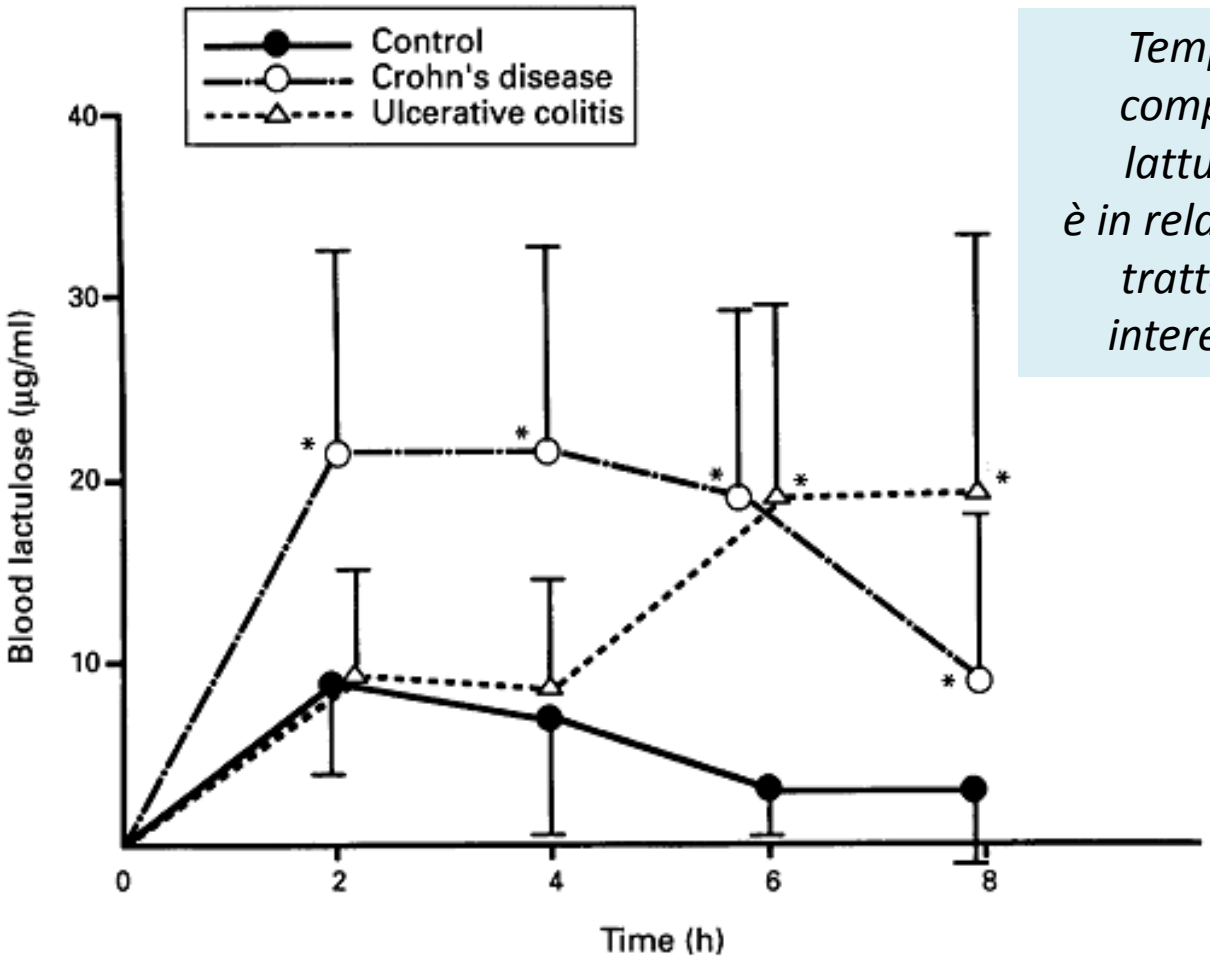


*Figure 2: Changes in the blood lactulose concentrations (mean (SD)) after oral administration in patients with Crohn's disease (n=22) and ulcerative colitis (n=19) compared with the control subjects (n=12). Blood lactulose concentrations significantly increased at all time points in patients with Crohn's disease and at six and eight hours in ulcerative colitis v controls. *p<0·001 v controls (Mann-Whitney U test).*

Tempo di comparsa lattulosio è in relazione al tratto GIT interessato

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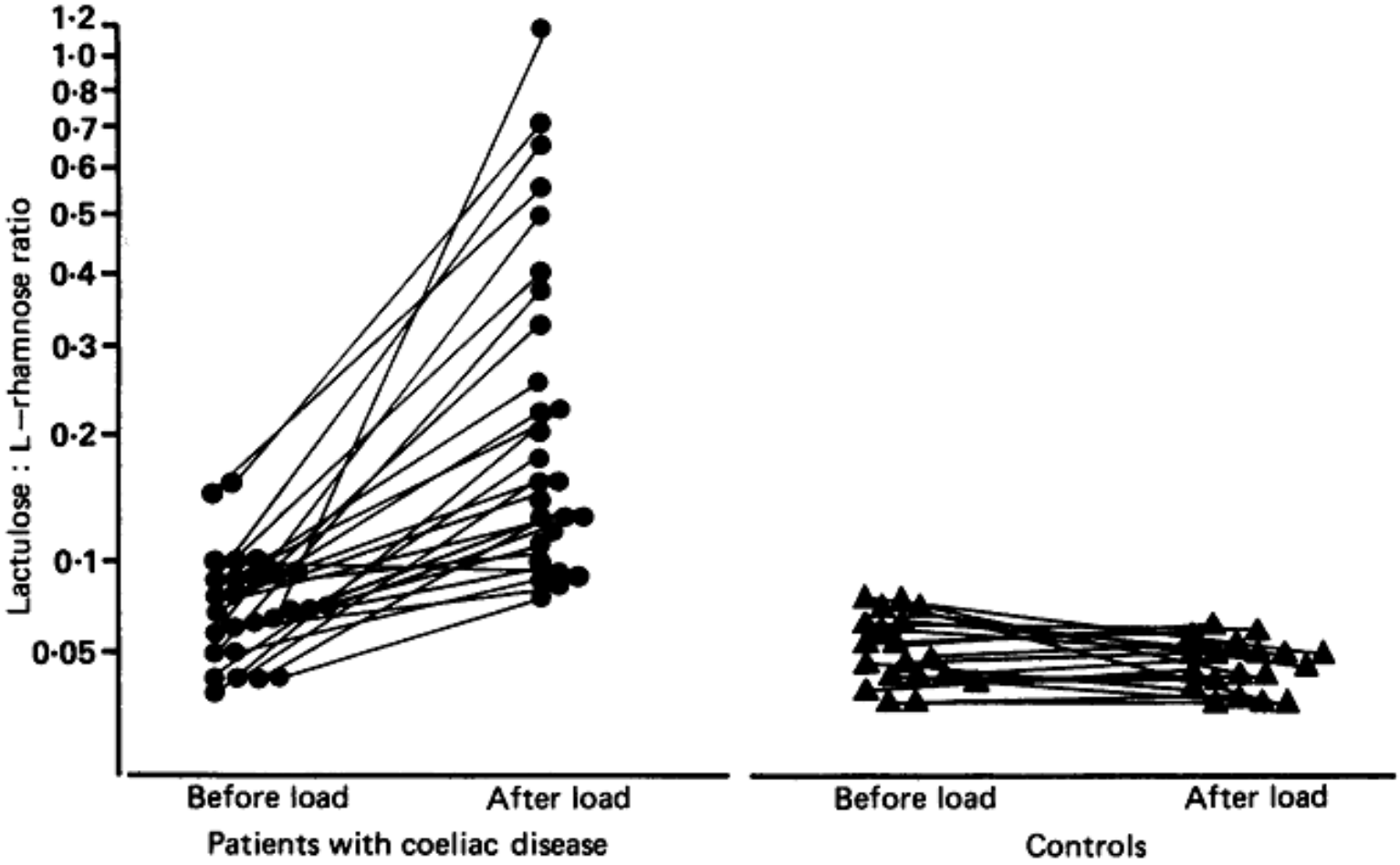
Rapporto lattulosio/ramnosio ematico in soggetti celiachi e sani, prima e dopo la somministrazione di una piccola quantità di glutine



Intestinal permeability following the 1998 Ironman triathlon.

Lambert, et. al. 1999.

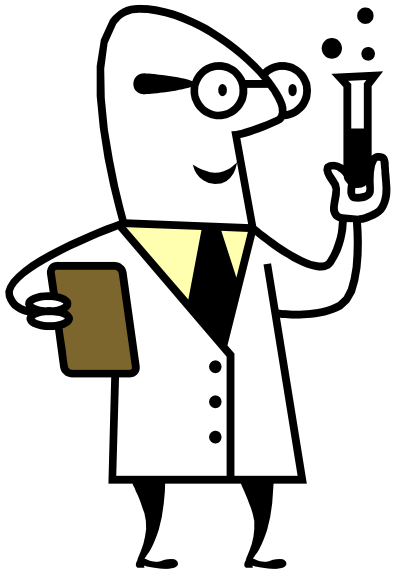
~ 3.8 km nuoto in mare;

~ 179- km ciclismo;

~ 42- km corsa.

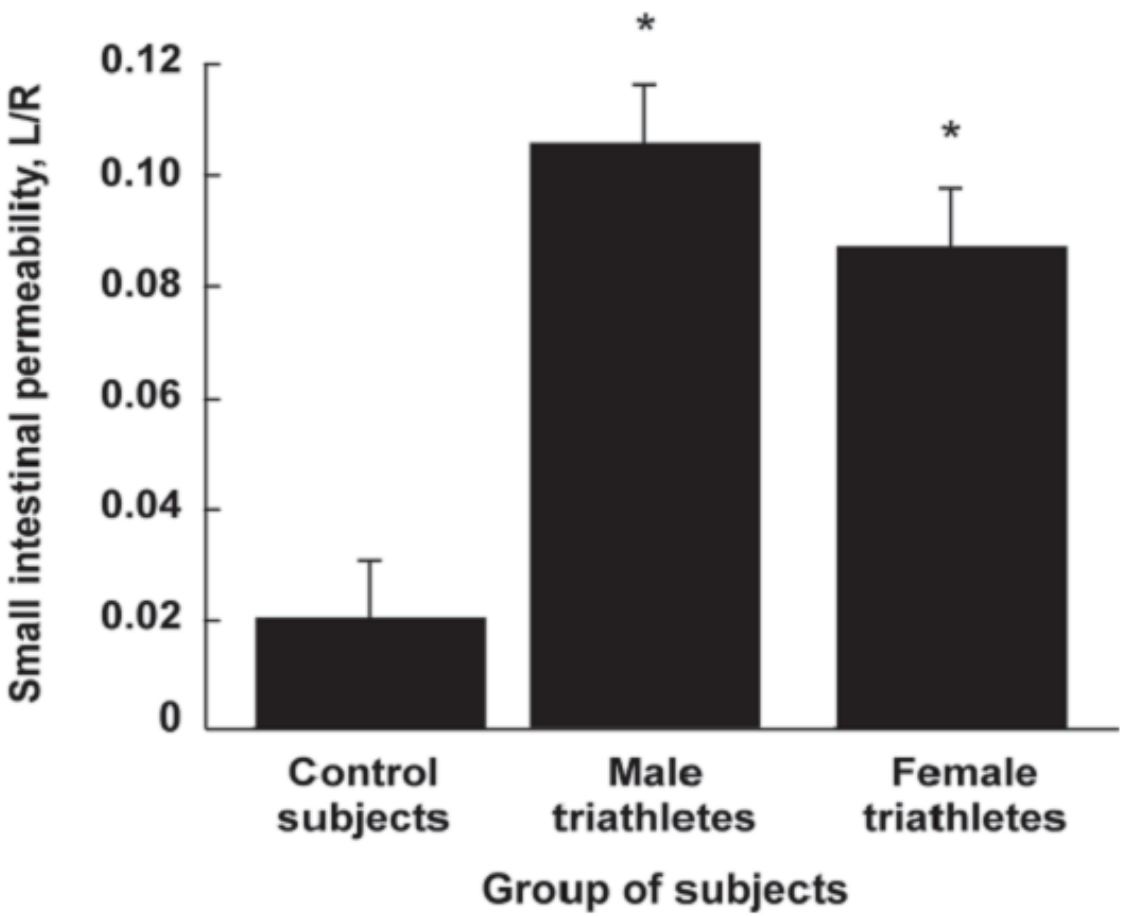
→ Lattulosio

→ Analisi urine



Attenzione Dott. Paglia ….

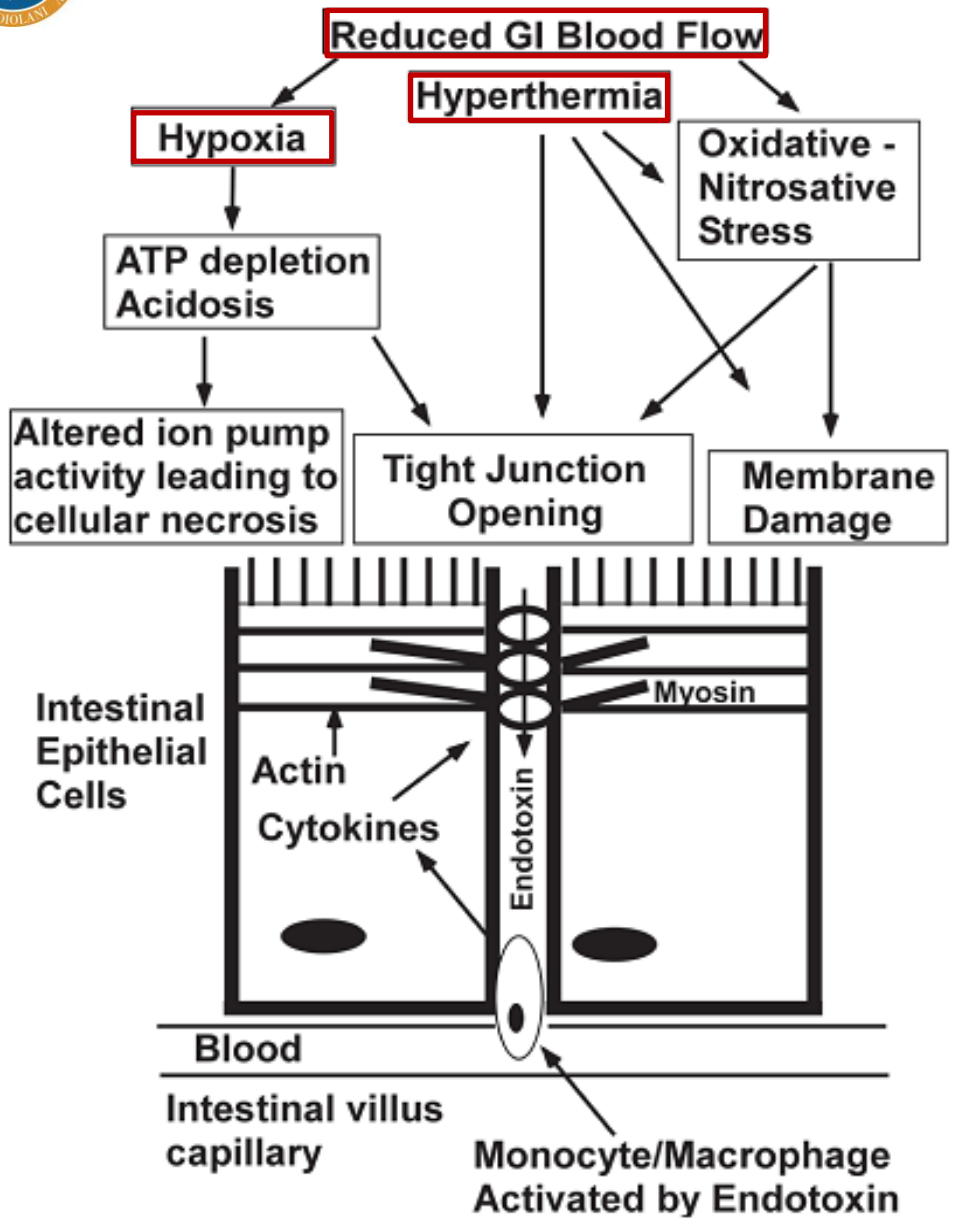
Risultati di Lambert

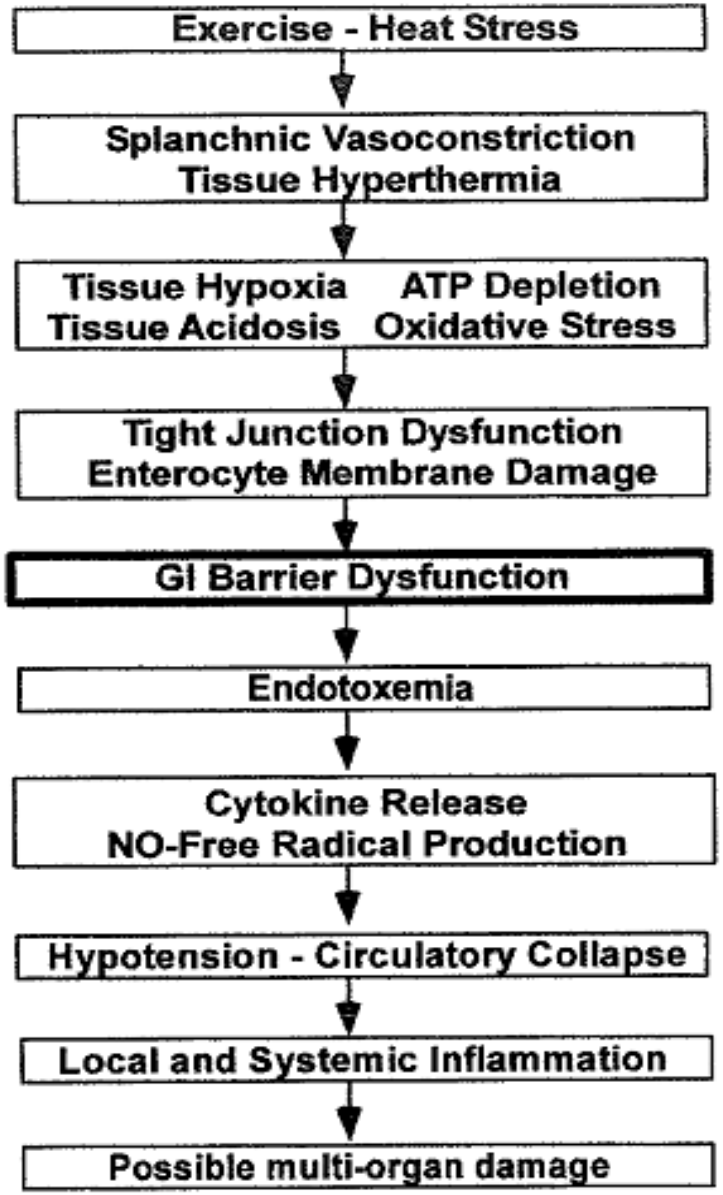


Permeabilità intestinale in atleti

Lambert et al., 1999 Med. Sci. Sports Exerc. 31:S318

Risultati di Lambert





Lambert, 2009, J ANIM SCI 87:E101-E108

Cause di alterata permeabilità

- Stress
  - Heat
  - Physical
  - Psychological
  - Ischemic
  - Trauma – burns, surgery
- Nutrition and Diet
  - Malnutrition of the gut
  - Nutrient status
    - Vit. D, Zinc, antioxidants
- Immune status
  - Immune activation and inflammation
  - Immunocompromise
- Exercise
- Age
  - Young and old
- Genetics
  - NOD2 (IBD)
- Drugs
  - Anti-inflammatories
  - Antibiotics
- Disease
  - Infectious
    - Bacterial, viral, protozoal
  - Non-infectious
- Pancreatic function

Stressori e permeabilità intestinale

POSSIAMO MISURARLA nei ruminanti?

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Permeabilità Intestinale

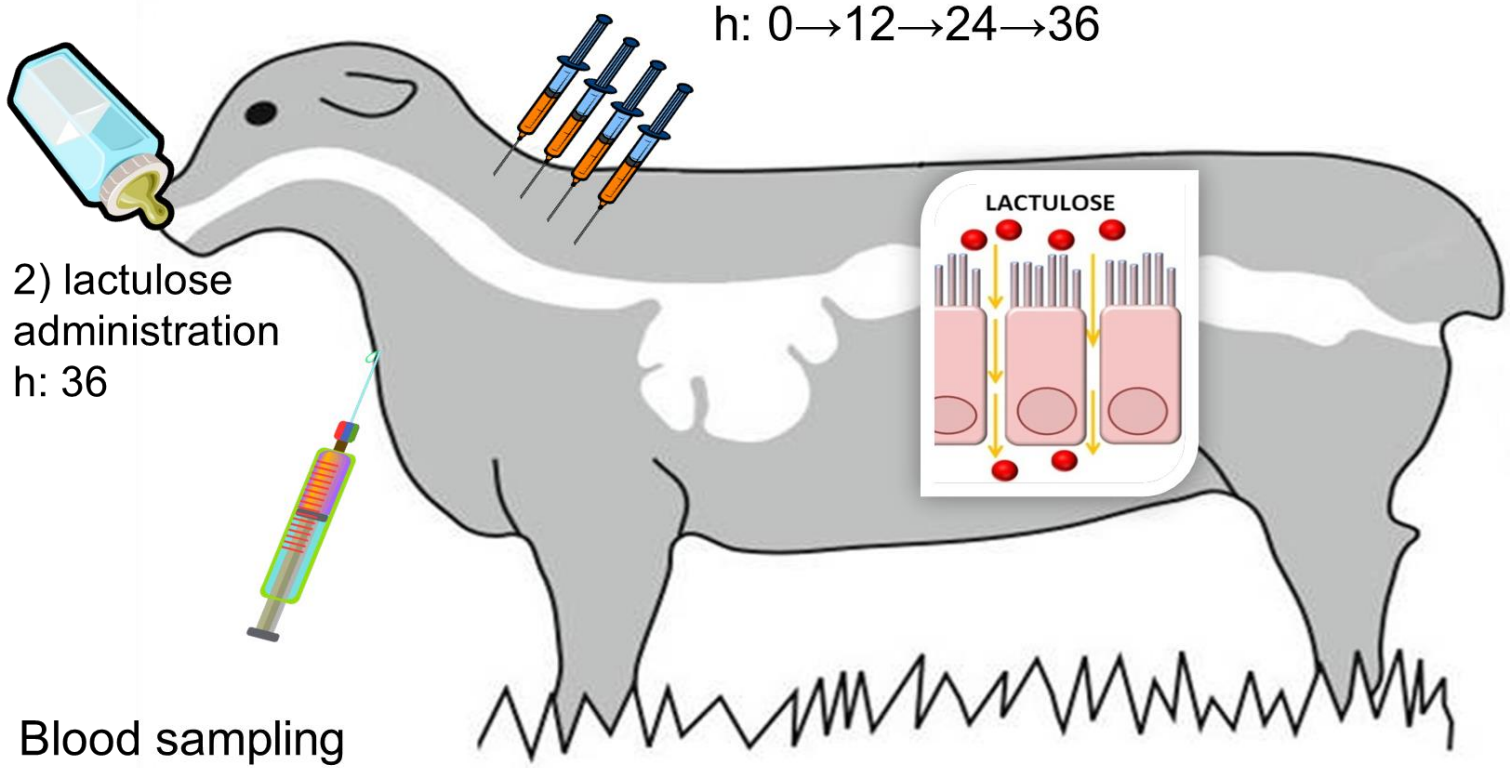
JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Assessment of gastrointestinal permeability by lactulose test in sheep after repeated indomethacin treatment

A. Minuti, S. Ahmed, E. Trevisi, F. Piccioli-Cappelli, G. Bertoni and P. Bani

Minuti et al., *Journal of Animal Science* 2013, 91:5646-5653.

Permeabilità Intestinale



Blood sampling
h: -12→12→36→60→132→204 (inflammatory status evaluation)

38→40→42→44→46
lactulose recovery

Minuti et al., *Journal of Animal Science* 2013, 91:5646-5653.

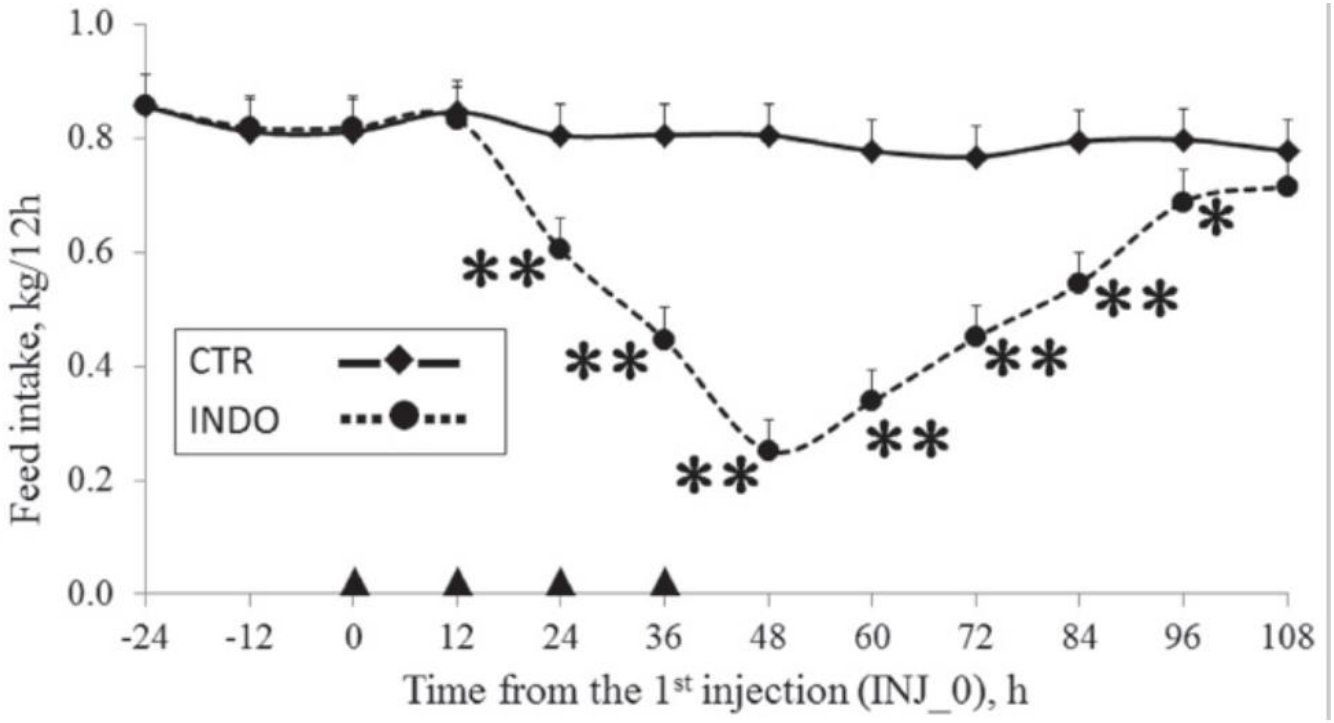
Health status & Feed intake

Clinical Symptoms in INDO Group

Dullness, weakness and depression

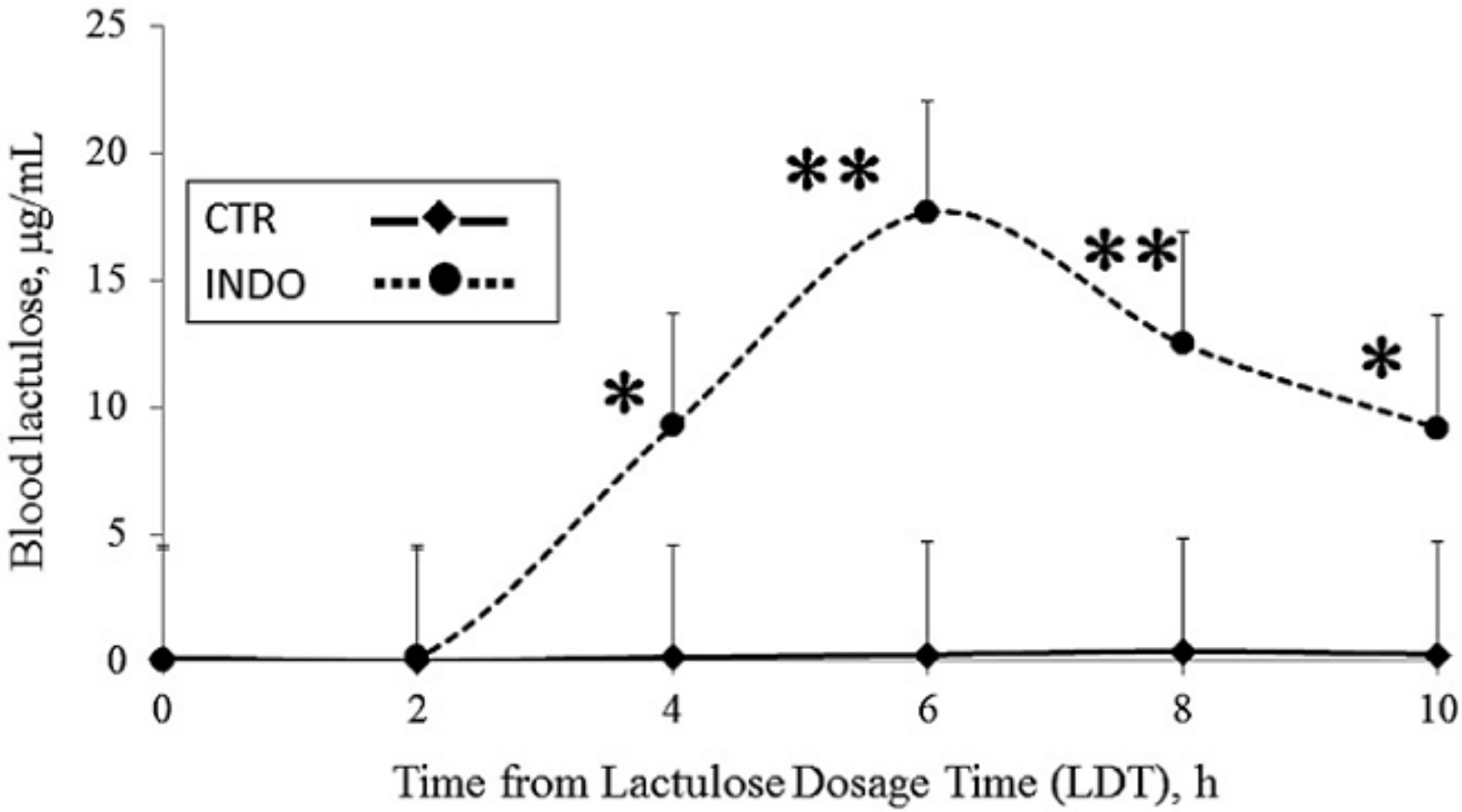
Soft stool just at 12h, then diarrhoea at 24h with some traces of blood

Drop of feed intake



Minuti et al., *Journal of Animal Science* 2013, 91:5646-5653.

Lattulosio nel sangue



Pattern of changes (average ± SD) of plasma lactulose concentration (µg/ml) after oral administrationin

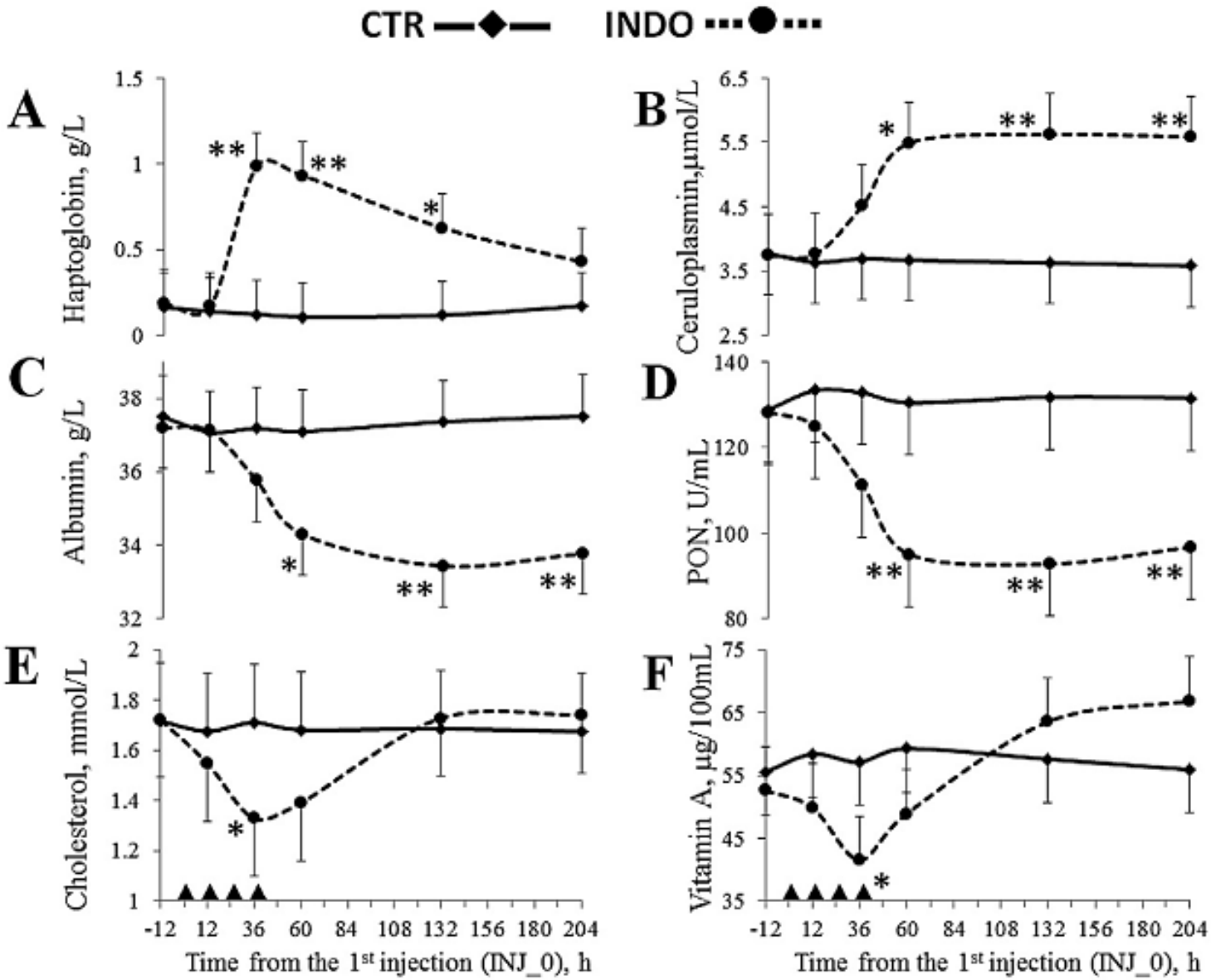
Minuti et al., *Journal of Animal Science* 2013, 91:5646-5653.

Conseguenze - Infiammazione

CTR (—◆—)
INDO (···●···) animals

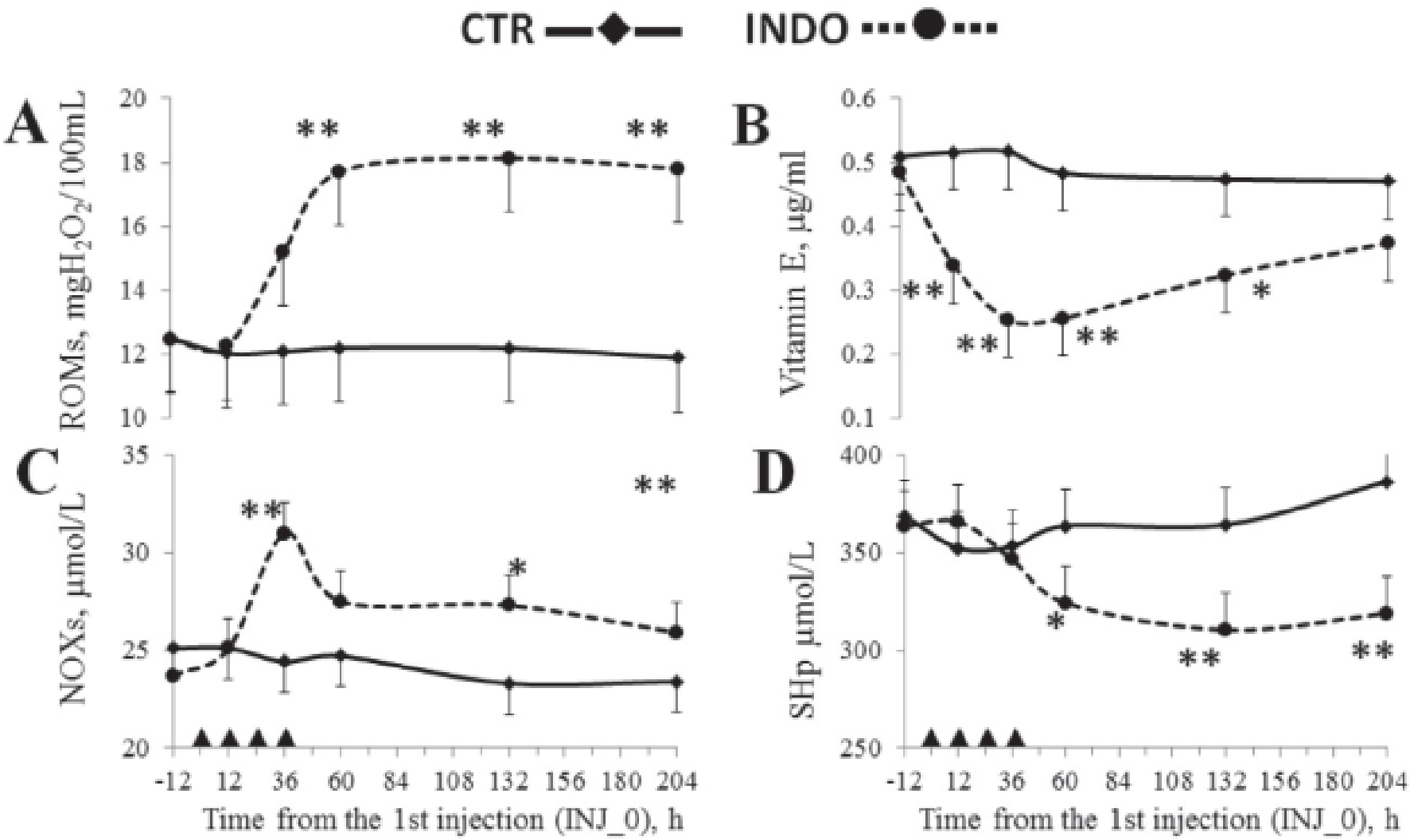
The triangle (▲) indicates the injection schedules of indomethacin

Significance of differences between groups at each time point is indicated by * (P<0.05); ** (P< 0.01).



Minuti et al., *Journal of Animal Science* 2013, 91:5646-5653.

Conseguenze – Stress ossidativo

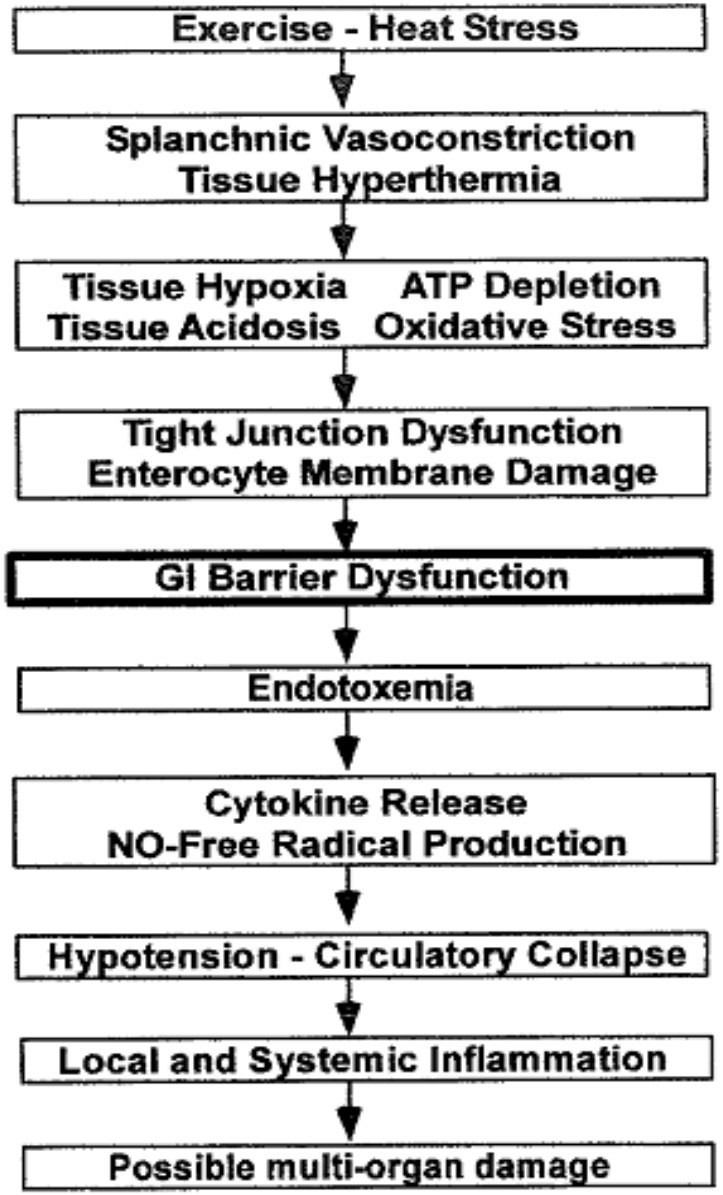


Minuti et al., *Journal of Animal Science* 2013, 91:5646-5653.

Implicazioni

I fattori che aumentano la permeabilità intestinale dei ruminanti sono responsabili di:

- ✓ malessere,
- ✓ febbre
- ✓ riduzione dell'appetito (e performance)
- ✓ disturbi metabolici
- ✓ gravi disturbi del sistema immunitario



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Acidosi acuta - indigestione

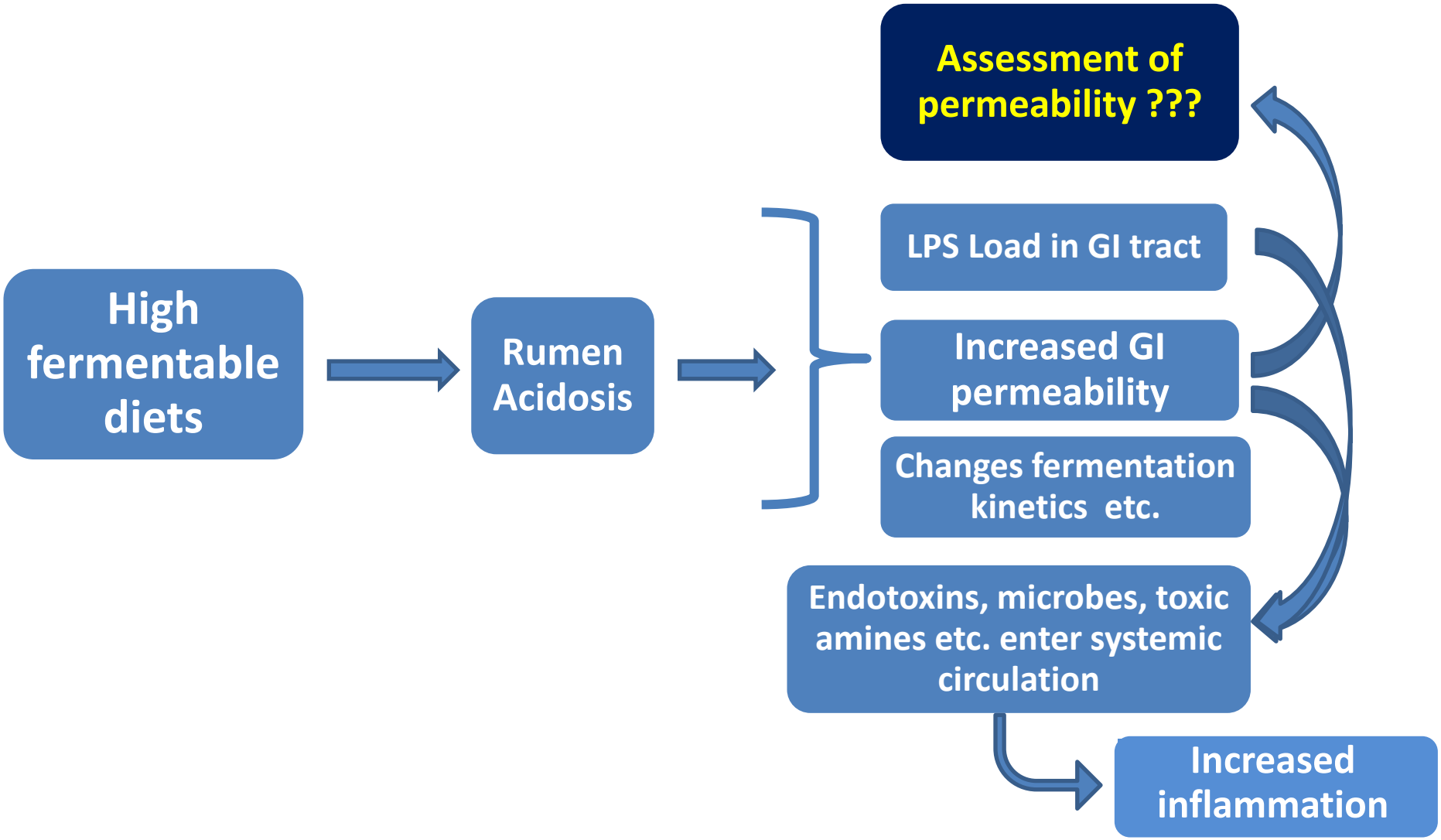
JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Experimental acute rumen acidosis in sheep: Consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry

A. Minuti, S. Ahmed, E. Trevisi, F. Piccioli-Cappelli, G. Bertoni, N. Jahan and P. Bani

Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977.

Acidosi acuta - indigestione

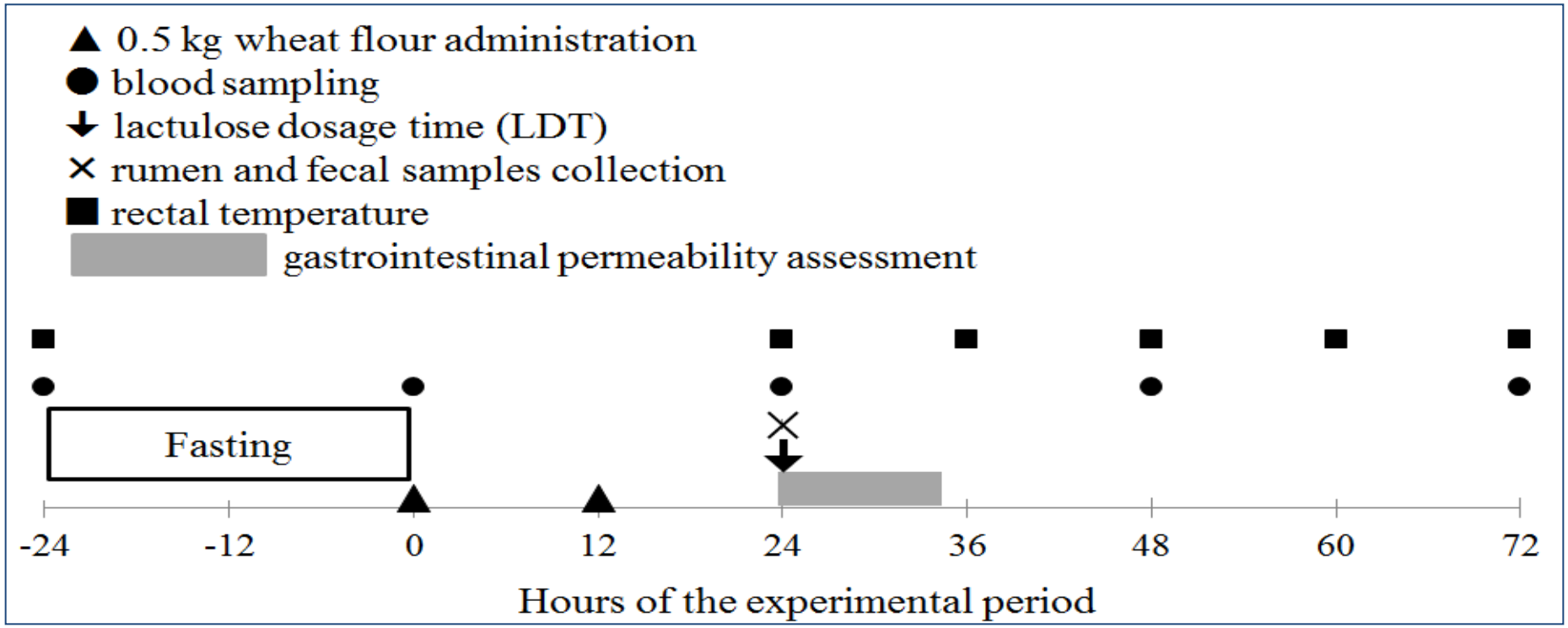


Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977.

Methodology

Monitoring and Blood analysis:

- **Clinical symptoms, feed intake and rectal temperature**
- **wide range of metabolic parameters**
- **lactulose level in blood**



Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977

Clinical Symptoms

In ACID group

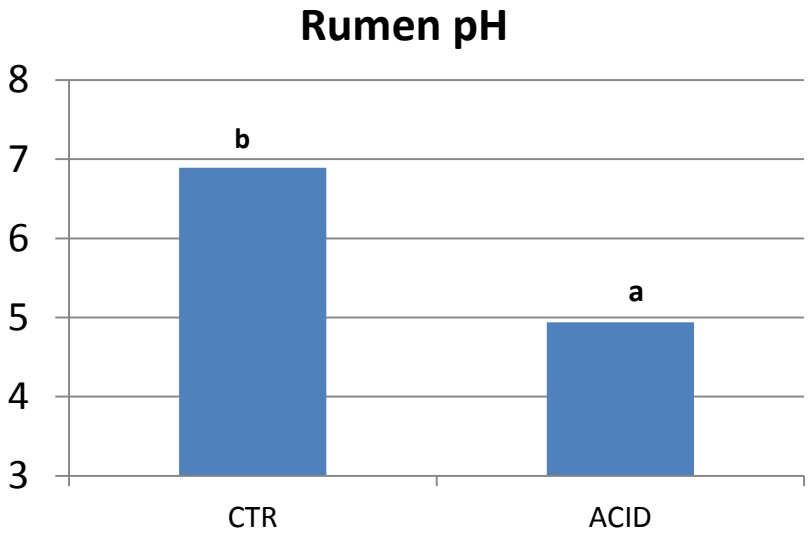
- Increase of rectal temperature (P<0.01)
- CNS depression
- trembling
- watery, yellowish and acidic smelling diarrhea
- no appetite until 72h
- difficulty in walking

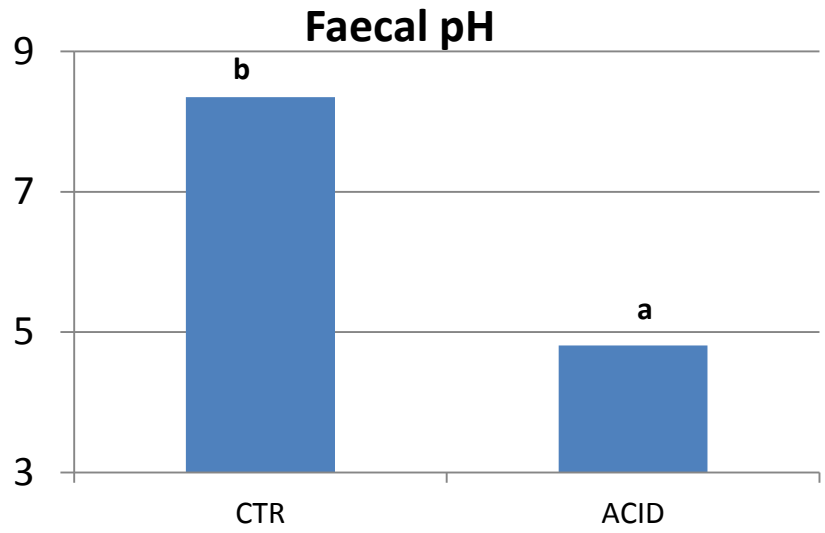
No clinical signs in CTR

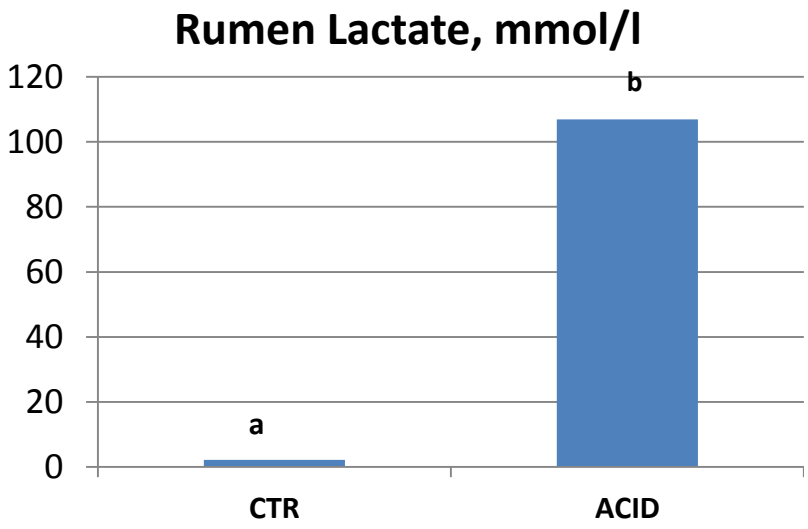
Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977

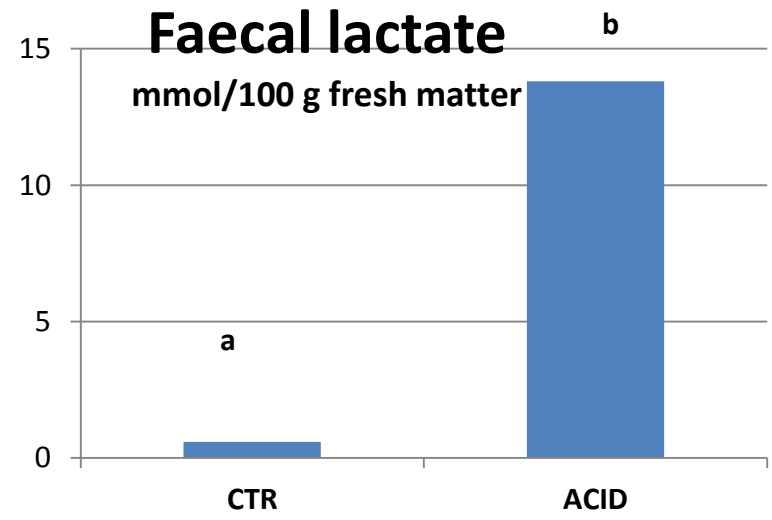
Conseguenze: rumine e feci

a, b; differ significantly (p<0.01)



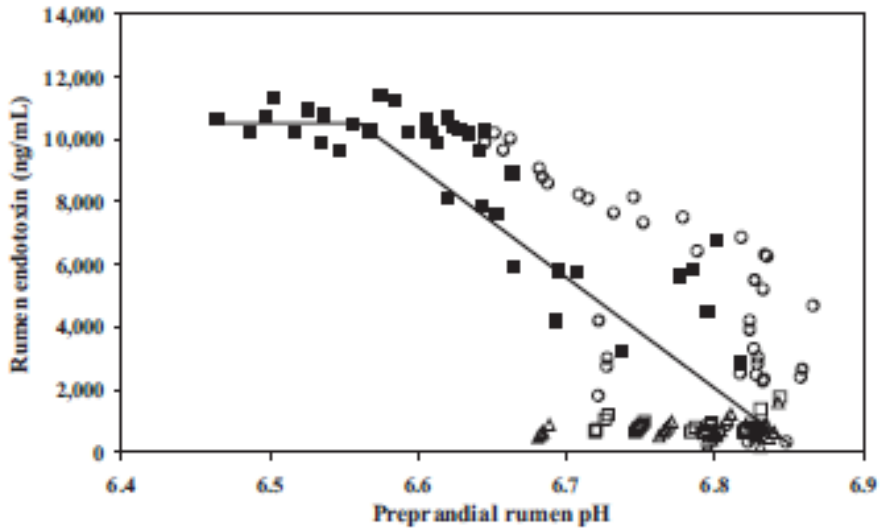




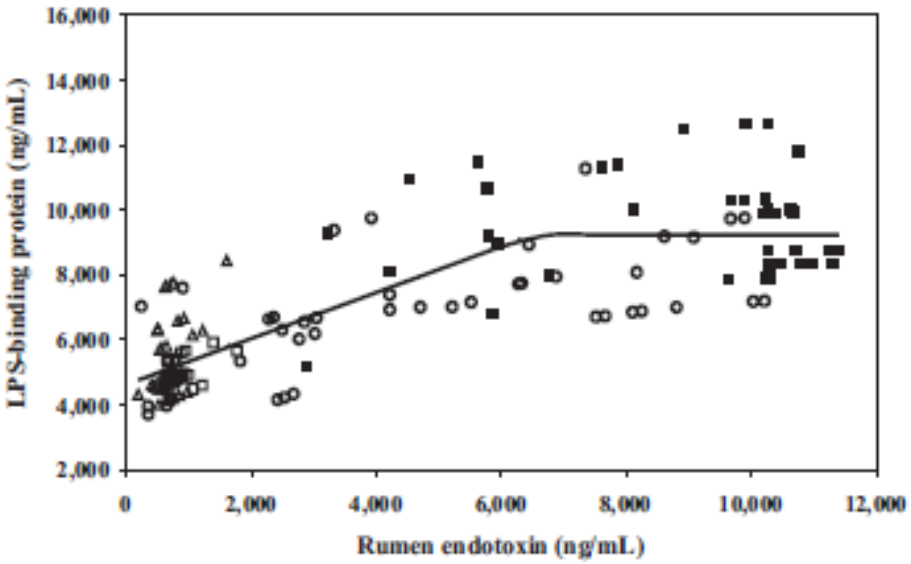


Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977

LPS diet & rumen pH



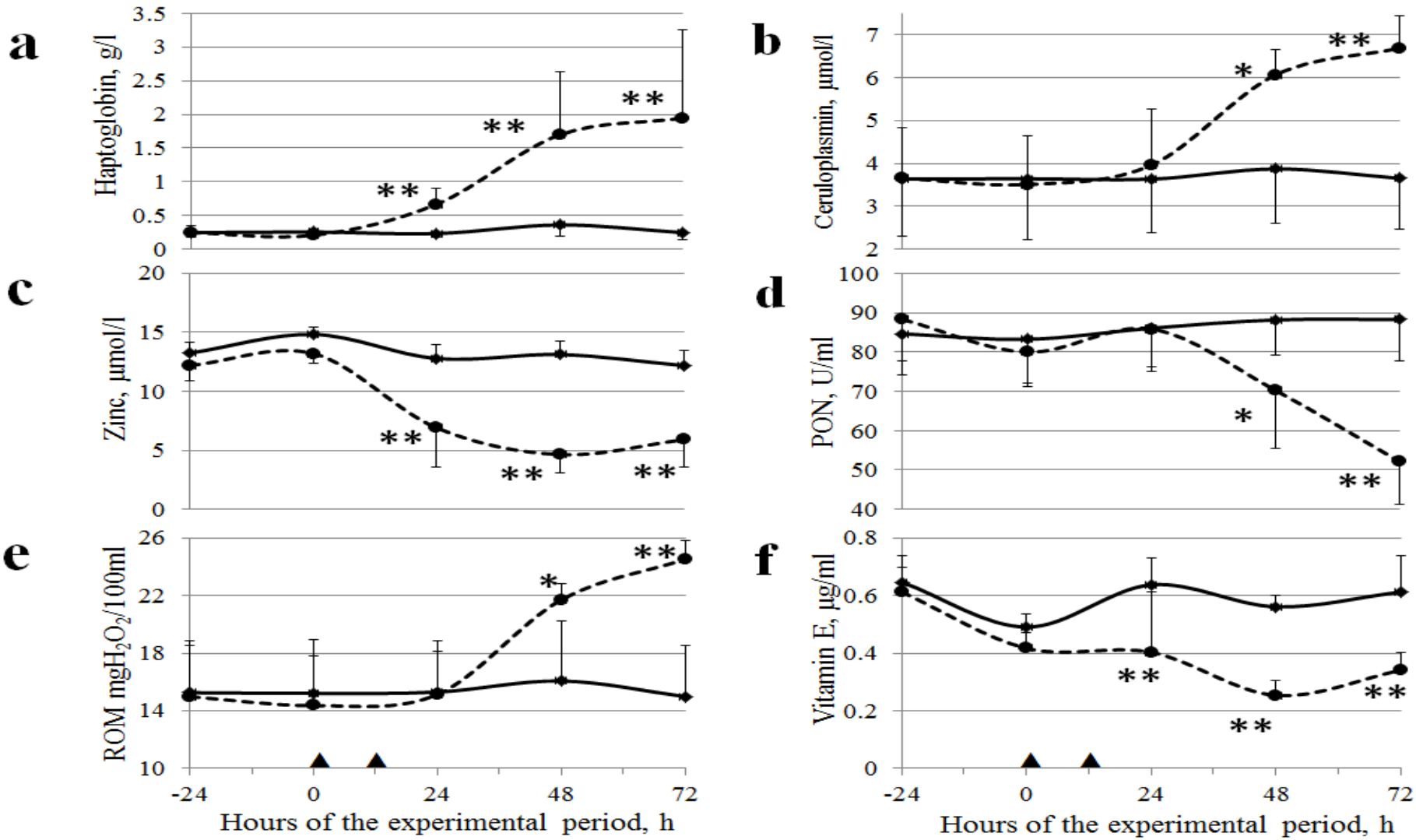
The **relationship between rumen pH and concentration of LPS in the rumen fluid** in dairy cows fed increasing amounts of rolled barley grain in the diet: strong negative relationship between preprandial rumen pH and concentration of LPS in the rumen fluid.
Rumen pH explained 64% in the variation of rumen LPS response.



The fitted model showed that **concentration of rumen LPS** was linearly **decreased** when rumen pre-prandial **pH exceeded** a value of **6.56** (Ametaj et al., 2010, R. Bras. Zootec., 39:433-444).

Emmanuel et al., 2008, *Journal of Dairy Science, 91:606-614*

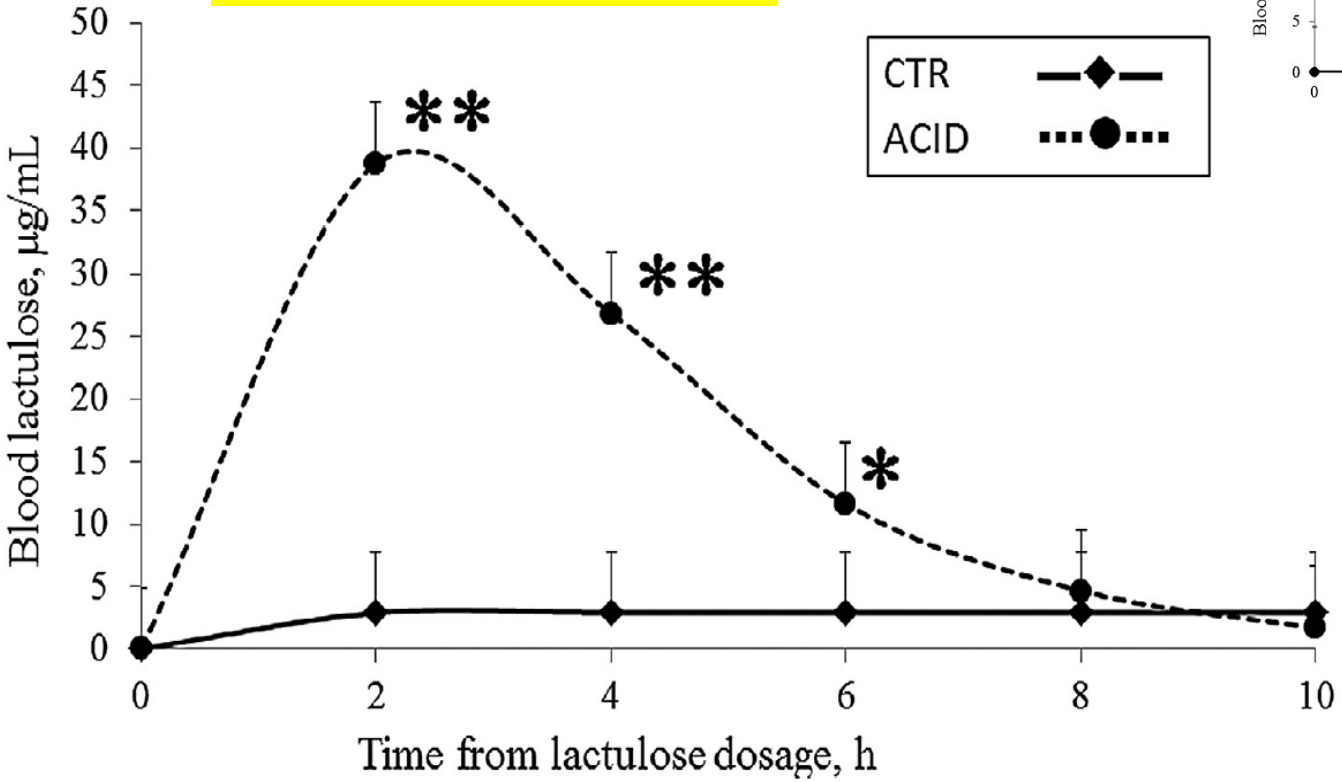
Plasma consequences

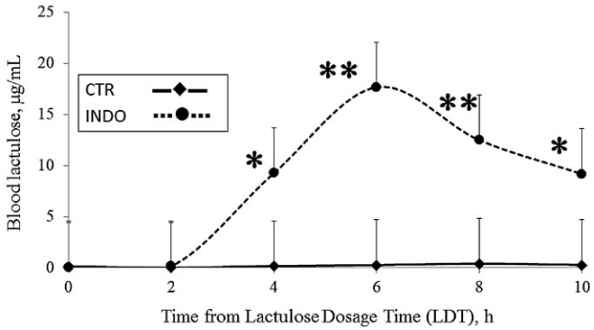


Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977

Permeabilità aumentata

…nel rumine?





Pattern of changes (average ± SD) of plasma lactulose concentration (µg/ml) after oral administration in CTR (—◆—) and ACID (···●···) animals. * (P<0.05); ** (P< 0.01)

Minuti et al., *Journal of Animal Science* 2014, 92:3966-3977

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Changes in the rumen epithelium in the transition period

J. Dairy Sci. 98:8940–8951
http://dx.doi.org/10.3168/jds.2015-9722
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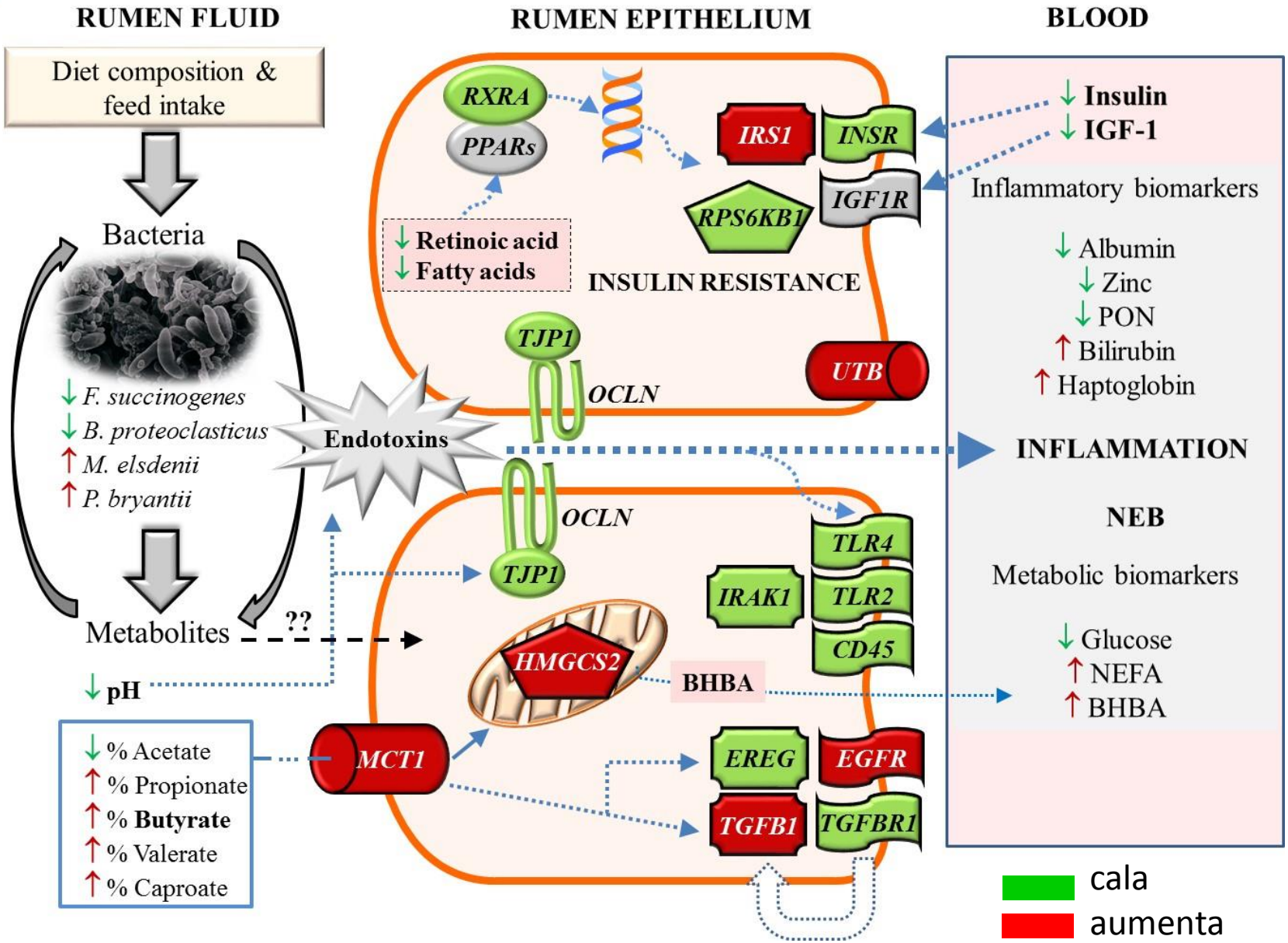
Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the peripartal period in dairy cows

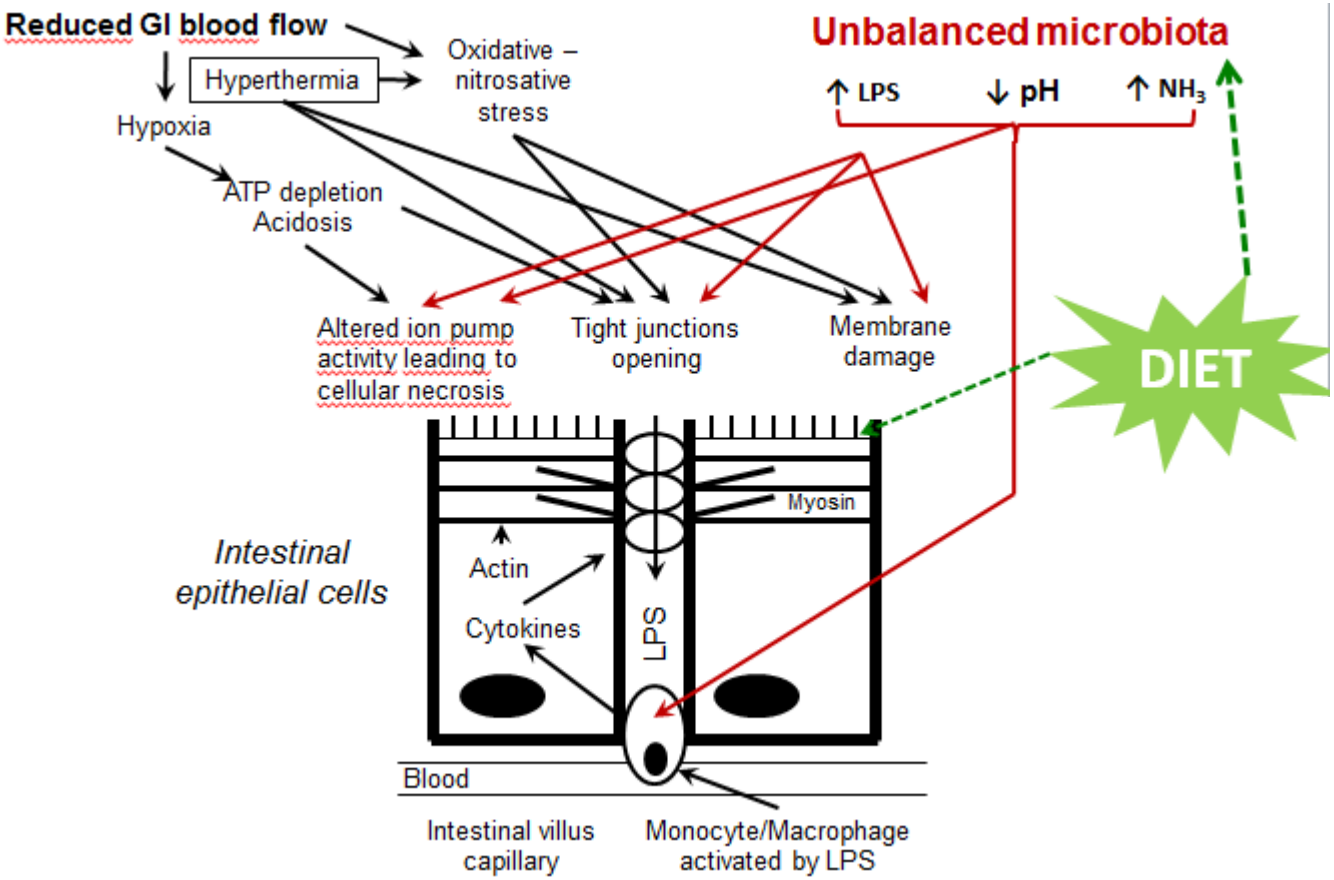
A. Minuti,* A. Palladino,† M. J. Khan,‡ S. Alqarni,‡ A. Agrawal,‡ F. Piccioli-Capelli,* F. Hidalgo,† F. C. Cardoso,‡ E. Trevisi,* and J. J. Loor‡[1]
*Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, 29122, Italy
†Department of Animal Production, Faculty of Agriculture, University of Buenos Aires, Av. San Martin 4454, 1417 Ciudad de Buenos Aires, Argentina
‡Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana 61801

Minuti et al., *Journal of Dairy Science* 2015, 98:8940-8951

LPS & rumen



Minuti et al., *Journal of Dairy Science* 2015, 98:8940-8951

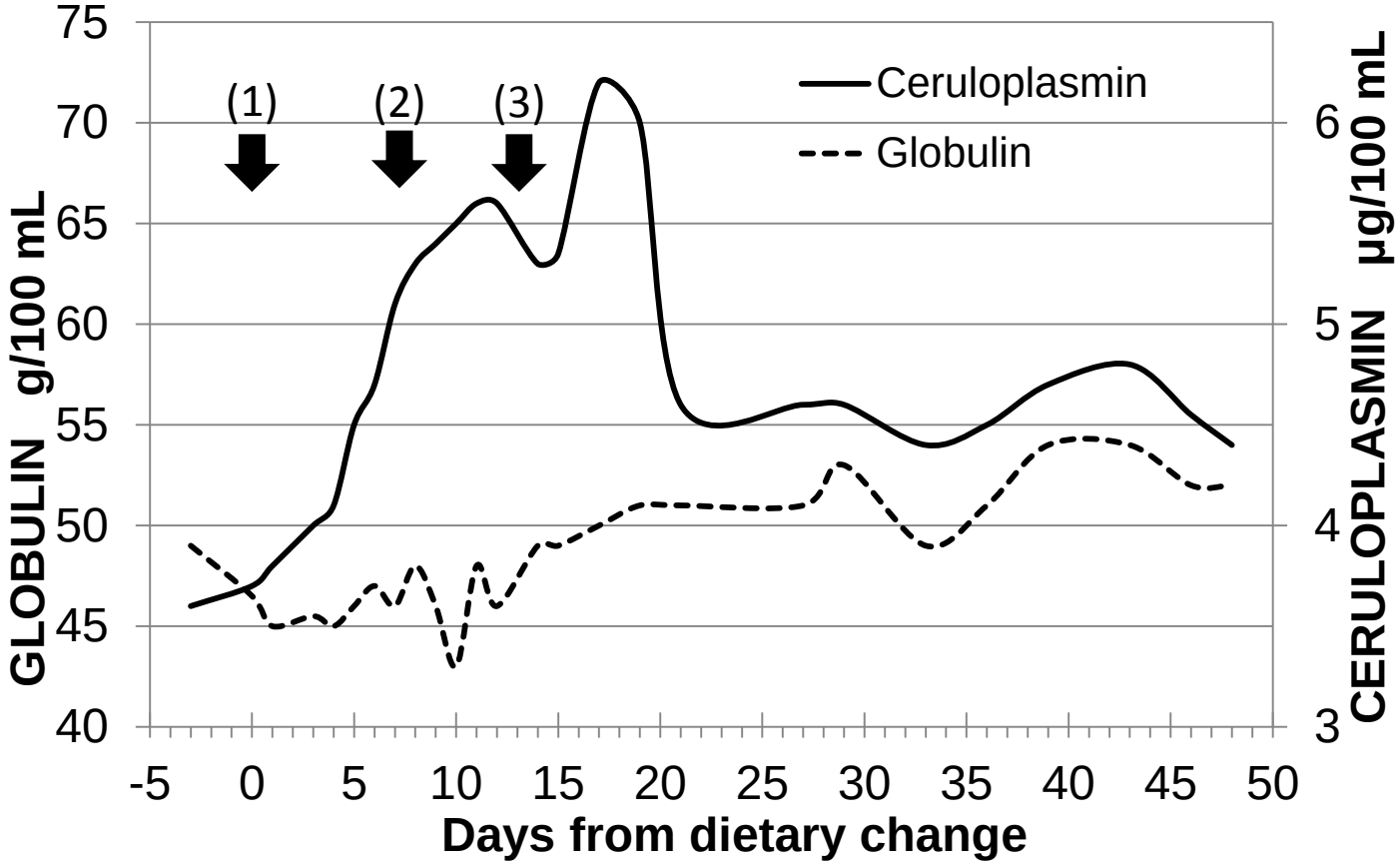


Damage of intestinal mucosa during severe exercise-heat stress, reduced intestinal blood flow and hyperthermia or during microbial overgrowth. GI, gastrointestinal; LPS, endotoxin. (Adapted from Lambert, 2009 and Cetin *et al.,* 2004).

Epitelio ruminale effetto del periparto (Postparto vs Preparto):

- ↓ Espressione geni delle tigh junction ☛ ↑ permeabilità
- ↓ Espressione geni implicati nella fz immunitaria ☛ ↓ difesa epitelio
- ↓ Espressione geni che «captano» glucosio ☛ insulinoresistenza

Changes in Diet and inflammation



Ceruloplasmin and globulins trends in the plasma of two ewes subjected to a sudden feed change (Calamari et al., 1980):

1) hay change with corn silage; 2) addition of 0,5 kg corn flour; 3) return to hay only

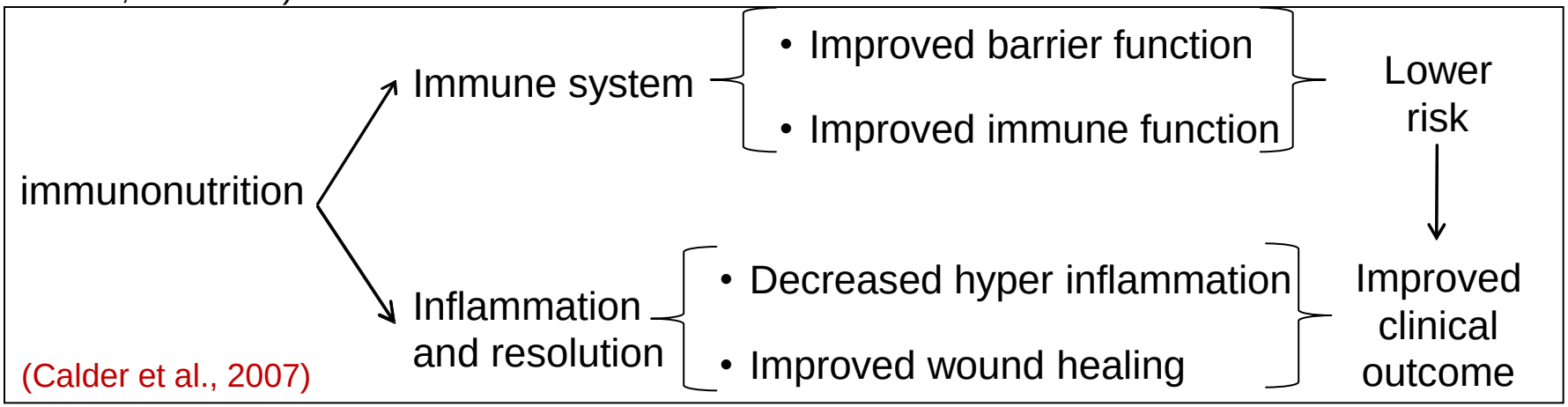
LPS: come contrastarle?

- ✓ **Prevenzione per ogni possibile causa di lesione (di ogni organo) o potenziale aumento di permeabilità degli epiteli con assorbimento di LPS (e LTA?)**
- ✓ Speciale cura nel periparto, dove LPS causano gravi complicazioni in tutte le tipiche patologie (a base infettiva e/o metabolica)
- ✓ Sviluppare meccanismi protettivi, in particolare, mantenere un sistema immunitario robusto
- ✓ Modulare la risposta infiammatoria (tempestiva ma breve)
- ✓ Migliorare approccio terapeutico (es. uso antibiotici può favorirne rilascio…. ed aggravare quadro clinico)

Ruolo essenziale della dieta

IMMUNONUTRITION

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



- **antioxidants**
- **some a.a. (sulphur)**
- **ω_3 fatty acids and CLA**
  - **ligands of PPARs**
  - **competitor of ω_6** and production of **eicosanoids** **with lower inflammatory power**
- **phyto-products (plants or some components)**
- **???**

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Dott.ssa Lovotti G.
Dott. Rossi D.
Sig. Dioli R.
e …..

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