Managing inflammation in dairy production: Phytogenics as a tool

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Euroopa Maaelu Arengu Põllumajandusfond: Euroopa investeeringud maapiirkondadesse

Digestarom® PFA solutions

- Introduction to phytogenic feed additives
- Different active compounds – different modes of action
- Understanding stress in cows
- BIOMIN – focus on gut health
- Discussion

What are phytogenic feed additives? How applied in animal nutrition?

The complexity of nature

Primary metabolites

Nutritive parameters

Necessary for plant growth

- Starch
- Sugar
- Oils
- Fiber

} CHOs

- Proteins/amino acids
- Vitamins/minerals

*Medicago sativa (*alfalfa/lucerne)

Source: Herbco.com

Secondary metabolites

Defense and dispersion

Pharmacolgical effects

- Odor (+/-)
- Taste (+/-)
- Pigments (+/-)
- Toxic deterents
- Bacteriostatic

Phytochemicals basis for phytogenic feed additives

Phytochemical:

- Refers to a variety of **plant-derived compounds with therapeutic activities** such as anticarcinogenic, antimutagenic, antiinflammatory and antioxidant properties (McGuire, 2011)
- **Diverse structure relates to bioreceptor interaction potential**
- Phytochemical composition varies by material, e.g. bark or leaves, or roots
  - Some compounds can by synthesised

Phytogenic feed additives (PFA) or botanicals:

- **Substances of plant origin** added to animal diets at recommended levels with the aim of improving animal wellbeing and performance
- Include whole plants, herbs/spice powders, volatile/non-volatile extracts, specific EO compounds

Natural variation in bioactive compounds

- World region/climate
- Season
- Environmental factors
  - Light
  - Temperature
  - Nutrients
  - Soil
  - Water
  - Pathogens
  - …
- Chemotype
- Development stage
- Plant parts used
- …

JOANNEUM RESEARCH

Institut für nachhaltige Techniken und Systeme

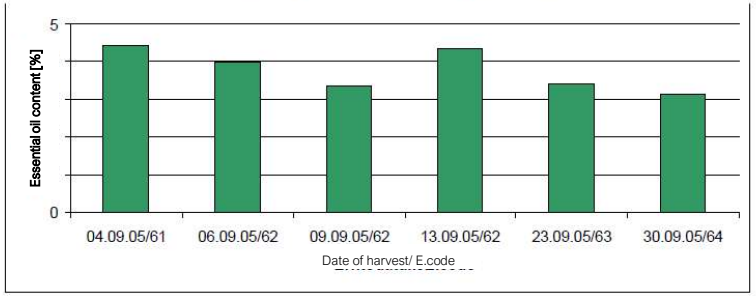
Oregano – Essential oil content and plant development
Results from 2005


E-code 51


E-code 59


E-code 61


E-code 64



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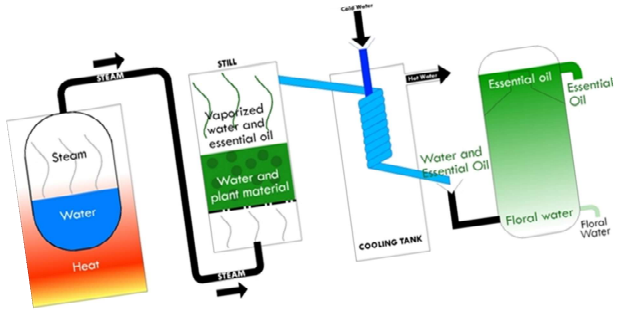
ISO 9001 zertifiziert

INNOVATION aus TRADITION

Specification of components

- Feed regulations
  - Commission Regulation (EU) No 68/2013
  - Regulation (EC) No 1831/2003
  - Regulation (EC) No 183/2005
  - Directive 2002/32/EC of the European Parliament and of the Council
  - FDA (US) GRAS status – code of federal regulations (CFR)
  - …
- Quality management requirements
  - GMP+
  - FAMI-QS
  - Pastus+
  - …
- Botanical identity and origin
  - Sensory properties
  - Chemical properties
  - Active ingredients
  - Essential oil
  - Total phenols
  - …
- Physical properties
  - Density
  - Viscosity
  - Optical rotation
  - Melting point
  - …



What about challenges in dairy production?

Understand the underlying cause, not the symptoms

A holistic view

- Invisible/Visible – Whole/Part
- Comprehensive review of Yang *et al*. (2015)

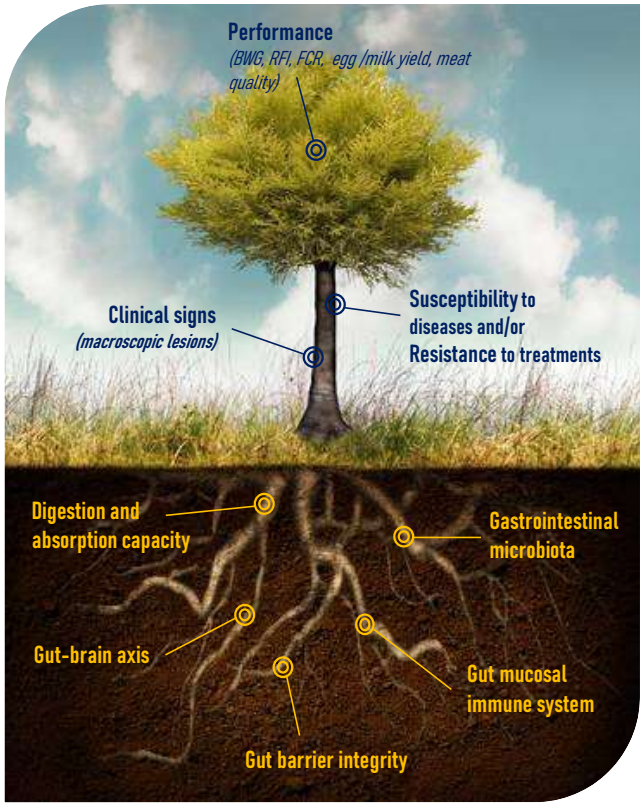
  Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. *Pathogens*, 4:137-156

  Microbiota: antimicrobial action, reduced VFA and ammonia, no antibiotic resistance

  Digestion: stimulation of enzyme activity, enhanced digestibility

  Gut-brain: improved feed palatability and intake (chemosensory system)

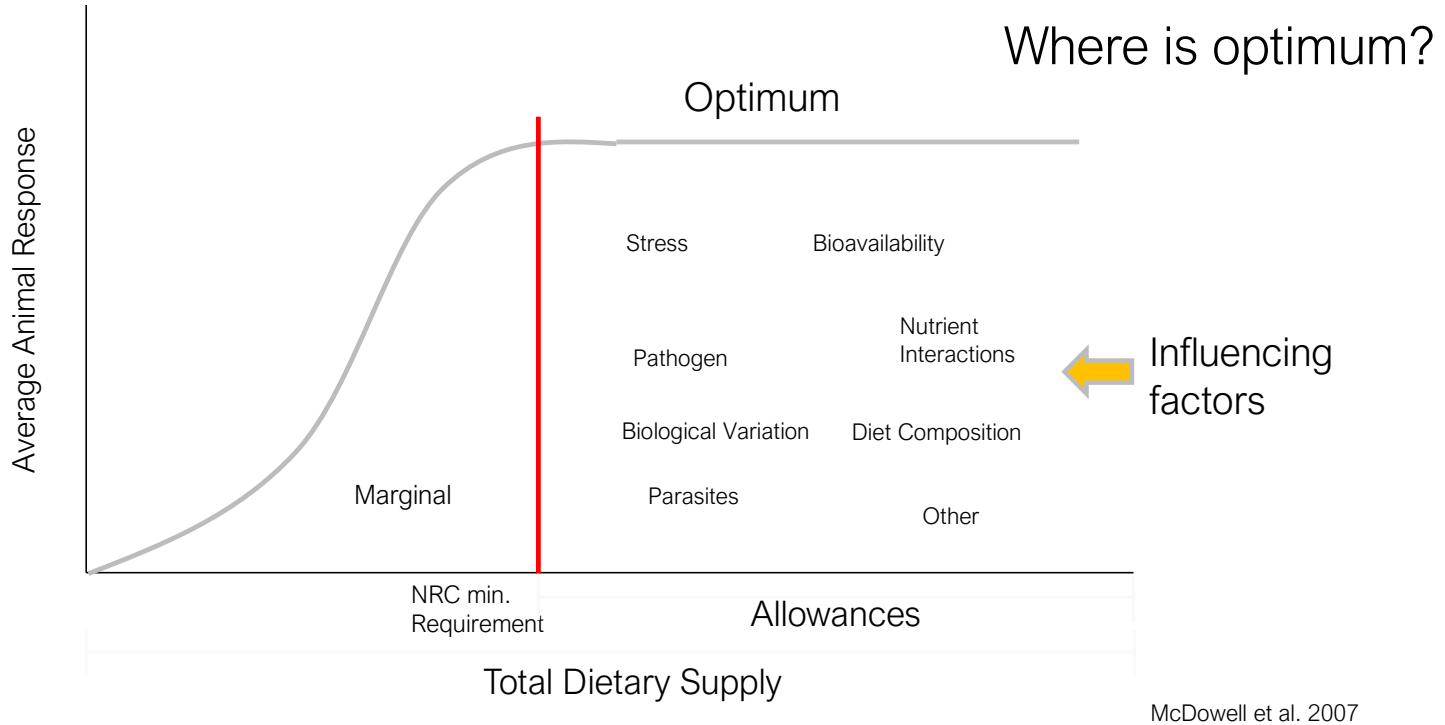
  Immunity: anti-inflammatory effects, oxidative stress defense, mucus secretion



"The **Way** we see the **things**"

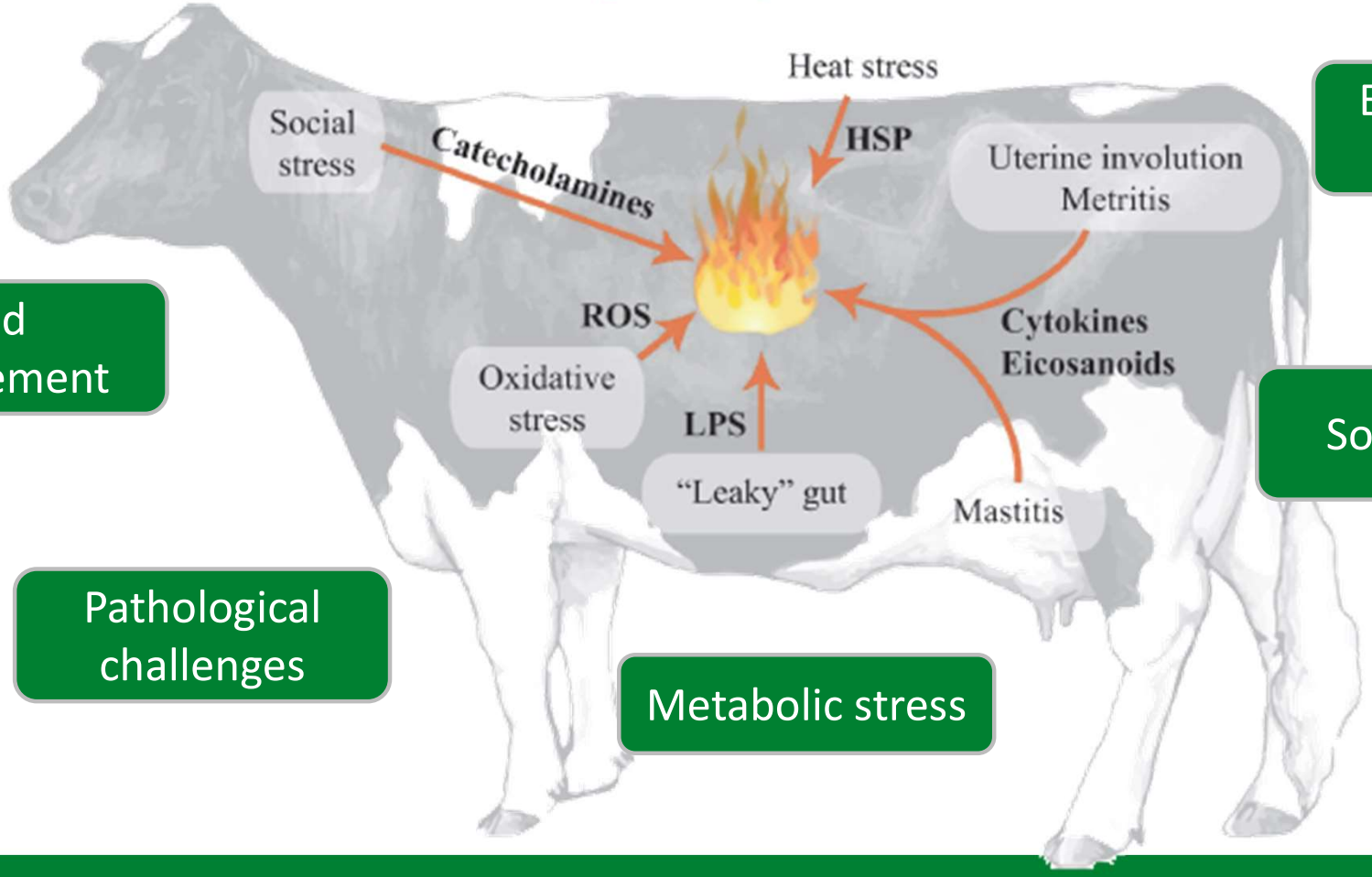
"The **Way** we should see the **things**"

Minimimum requirements ≠ optimum performance



McDowell et al. 2007

Inflammation in new perspective



Environmental stress

Feed management

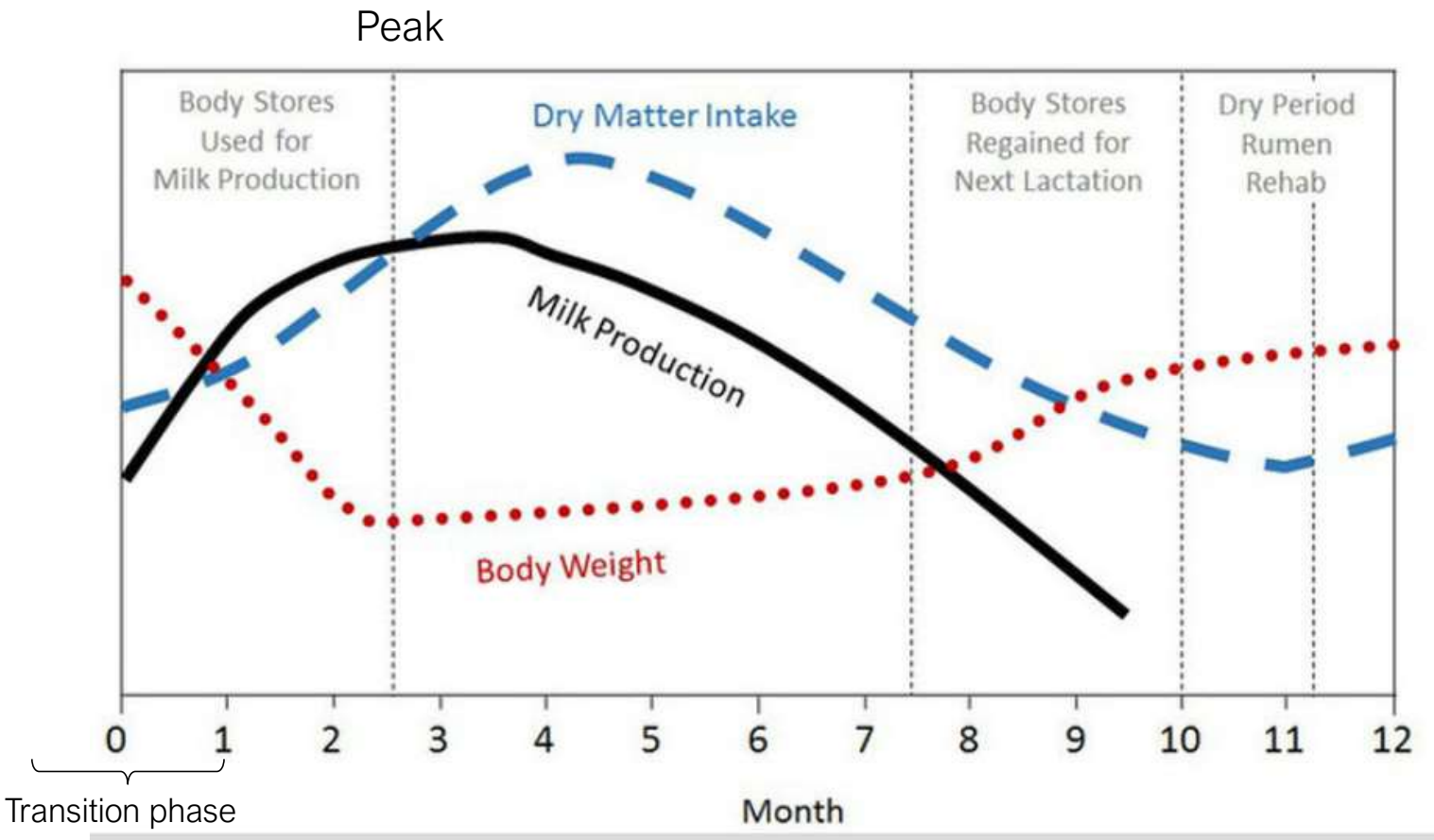
Social stress

Pathological challenges

Metabolic stress

Bradford et al. 2015

Challenges encountered throughout lactation



Metabolic disease prevalence

Metabolic challenges most prevalent in first 30 d post-calving

Disease	Clinical & Sub-clinical*
Ketosis	27% ± 9%
Metritis	25% ± 11%
Acidosis	26% ± 20%
Mastitis	8% ± 8%
Lameness	5% ± 3%

*Suthar et al. 2013 & Kleen et al. 2013

SCK

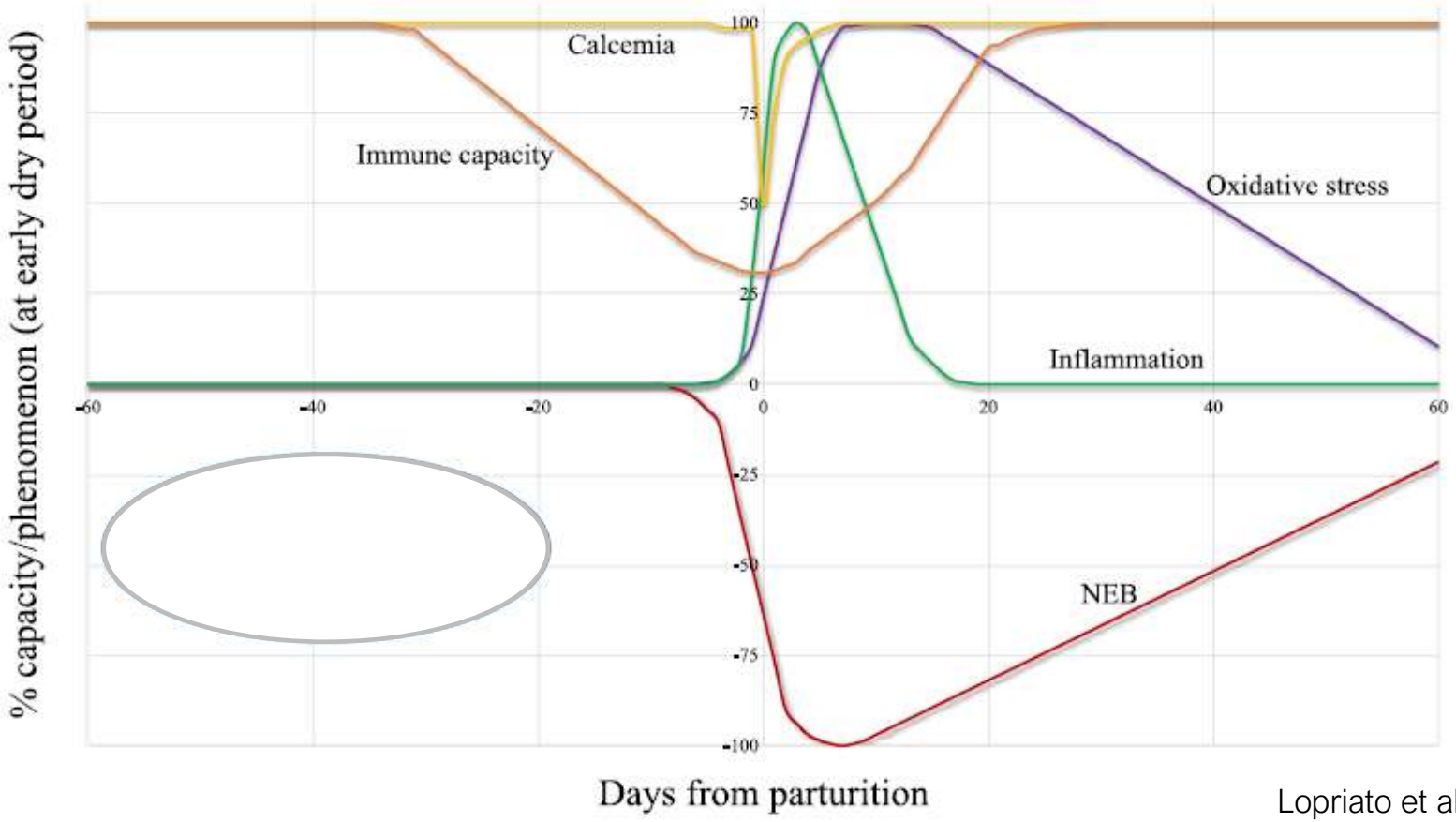
	Risk factor
Clinical ketosis	3 to 6x
LDA	2.6 to 8x
Metritis	1 to 5.8x
Milk Yield @ 160DIM	-1.5 to 2.4 kg/d
Delayed breeding	16 days

Leblanc 2010

Costs of metabolic disorder

- Direct treatment costs
- Indirect losses to milk production, depending on severity
- Increased susceptibility to multiple diseases
- Delayed fertility, widening calving intervals

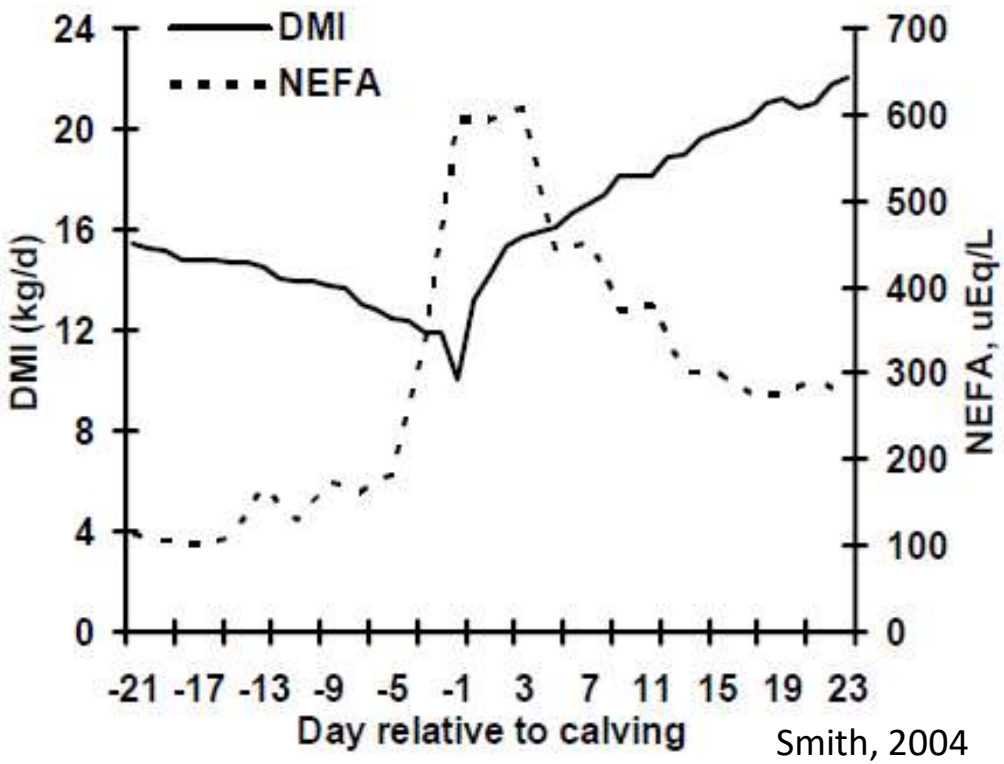
Finding balance during critical phase



Lopriato et al., 2020

Transition disorders begin before calving

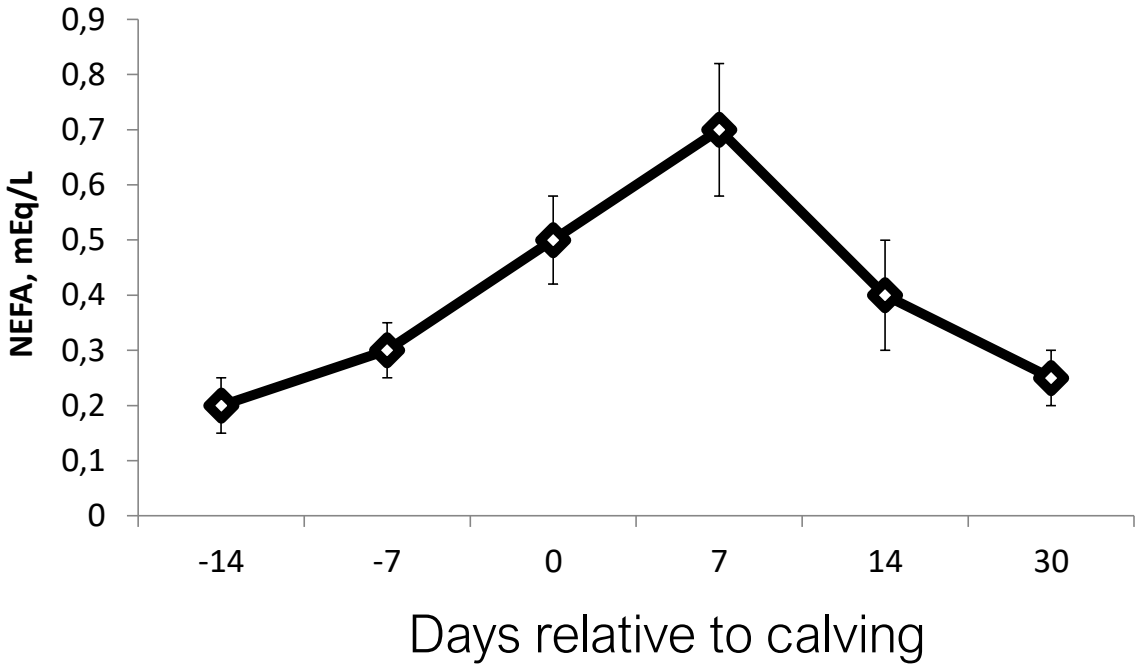
- DMI drives NEFA mobilisation
  - Catabolic reaction
  - Insulin resistance
  - Microbial disruption
  - Low microbial synthesis
  - Reduced rumen function
  - Low glucose circulation



Smith, 2004

Fat mobilization enhances inflammatory response

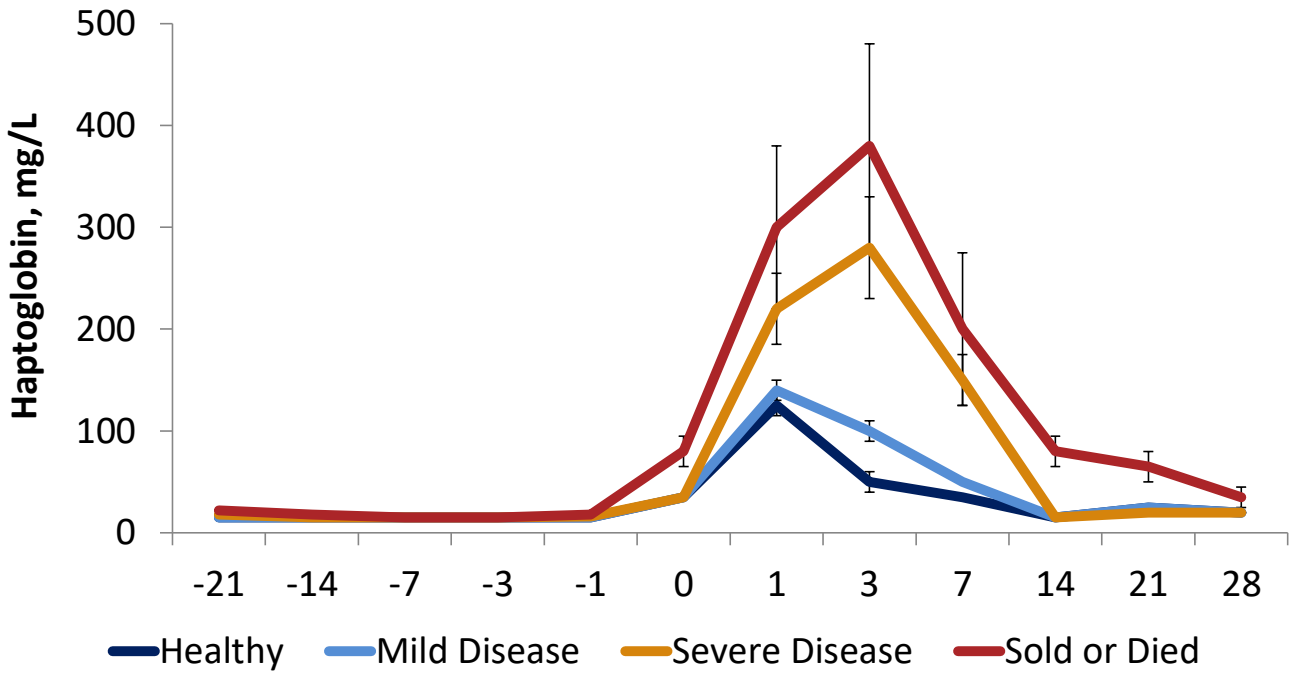
Body fat mobilization = Suppressed immune system



Contreras et al. 2010

Coping capacity determines inflammation threshold

Suppressed immune system = ↑ Inflammatory response



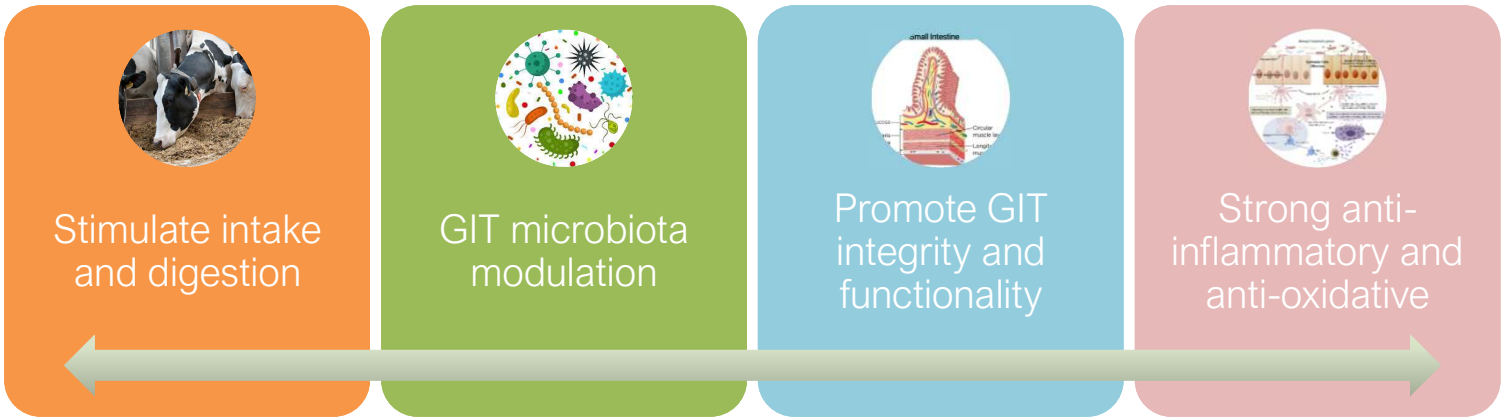
Sabedra 2012

Digestarom® Dairy

Role of phytogenic compounds in ruminant production

Phytogenics in animal production

Digestarom® product line is the solution to **improve nutrient availability** by **reducing sub-inflammatory processes**, modulating the gut microbiota and ensuring gut integrity for **better animals performance**

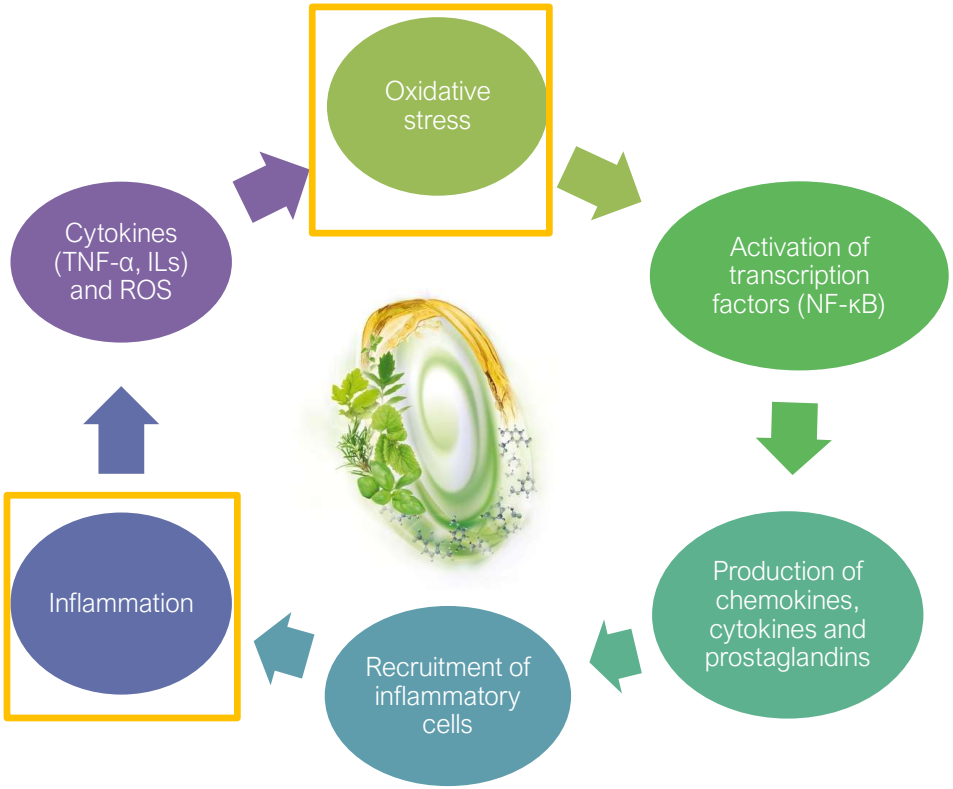


Gut health a complex interaction of multiple factors

Oxidative stress and inflammation – a viscious circle

↑ Stressors = ↓ Efficiency

Digestarom® operates on various steps of the vicious cycle to reduce the impact of oxidative stress and inflammation on animal performance



Adapted from: Mostofa et al., 2017

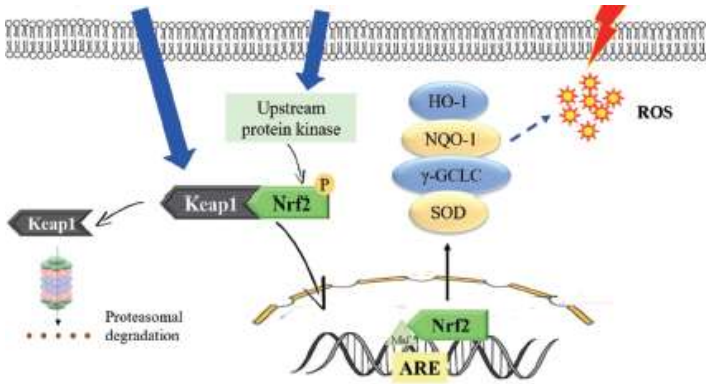
Metabolism and role of antioxidants

- Reactive oxygen species (ROS) generation
  - normal metabolic function in mitochondria (ATP formation)
  - oxidative burst defense by macrophages (anti-pathogen)
  - stress elevates ROS formation (cell metabolism)
  - damage occures when ROS formation exceeds coping capacity
  - Superoxide (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO)
    - Reactions can form additional reactive subclasses

- Many vitamins and minerals serve complementary antioxidant functions
  - Se, Zn, Mn, Cu, vit C, vit E, β-carotene
  - scavaging free radicals (Vit C, Vit E, β-carotene, phytophenols)
  - block lipid peroxidation of phospholipids (e.g. Vit E)
  - degrading hydrogen peroxide to H_2O (e.g. enzymes)

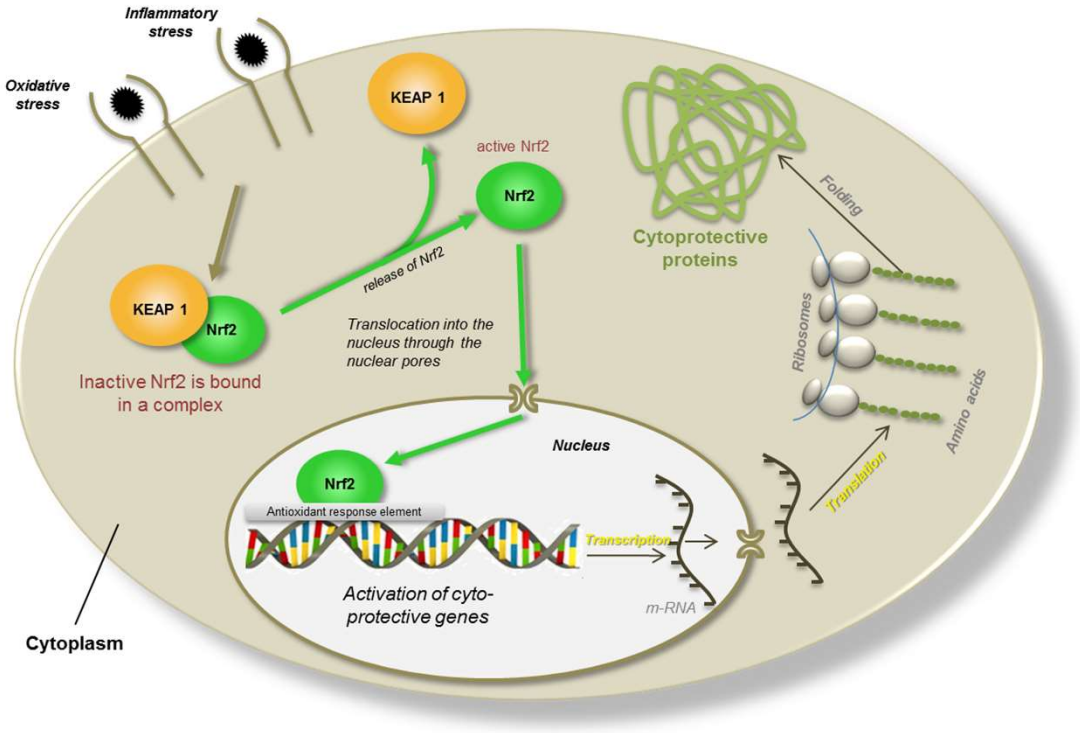
Phytochemicals as antioxidants

- Over 8000 known structural phytochemical variants
  - Antioxidant activity influenced by structure
    - Aromatic rings, hydroxyl moieties
    - Highly variable depending on region, climatic conditions and genetics (Chrysargyris et al. 2020)
    - Synergy enhances activity by 20-30% over single compounds (Hashemazadeh-Cigari et al. 2014)
  - Direct activity linked to superoxide scavaging activity
  - Indirect action via Nrf2 regulation (antioxidant response regulator)
    - Upregulates numerous enzyme defense responses, eg GST, CAT, SOD



Lee et al. 2017

Nrf2-system – Master regulator of cell protection



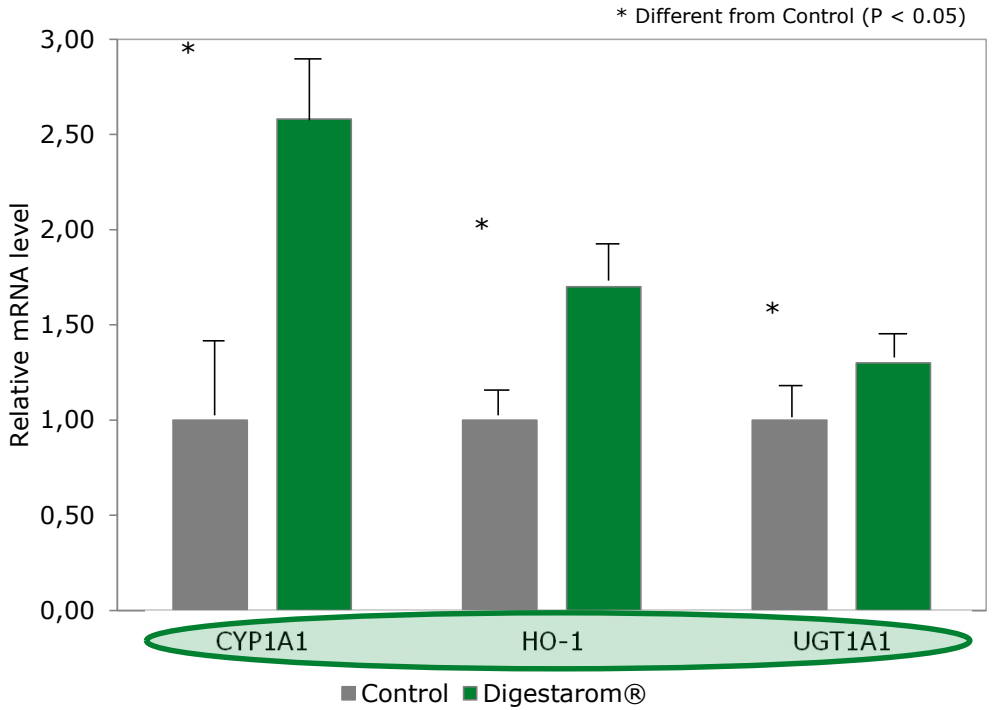
The Nrf2-system

- Nrf2 = **N**uclear factor E2-**r**elated **F**actor **2**
- The Nrf2-system is one of the major **cellular defense mechanisms** against oxidative & xenobiotic stresses in the intestinal tract.
- The Nrf2-system contributes to protection against various pathologies, including inflammation, liver toxicity, carcinogenesis & respiratory distress.

Enhancing expression of genes related to Nrf-2 (cell protection) with Digestarom®

In vitro test: Caco-2 cells

1. Cell control (Negative control)
2. Cell control + Digestarom®



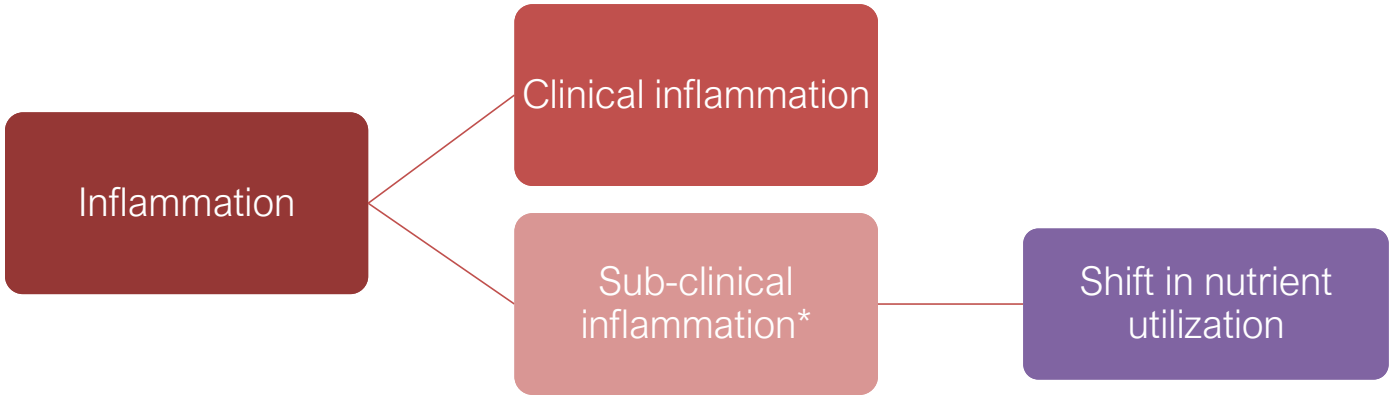
Gut protection markers

Enhancing cell protection

Source: University of Gießen, *unpublished data*

Impact of inflammation on performance

Inflammation - protective mechanism to coping with stressors and harmful environmental stimuli



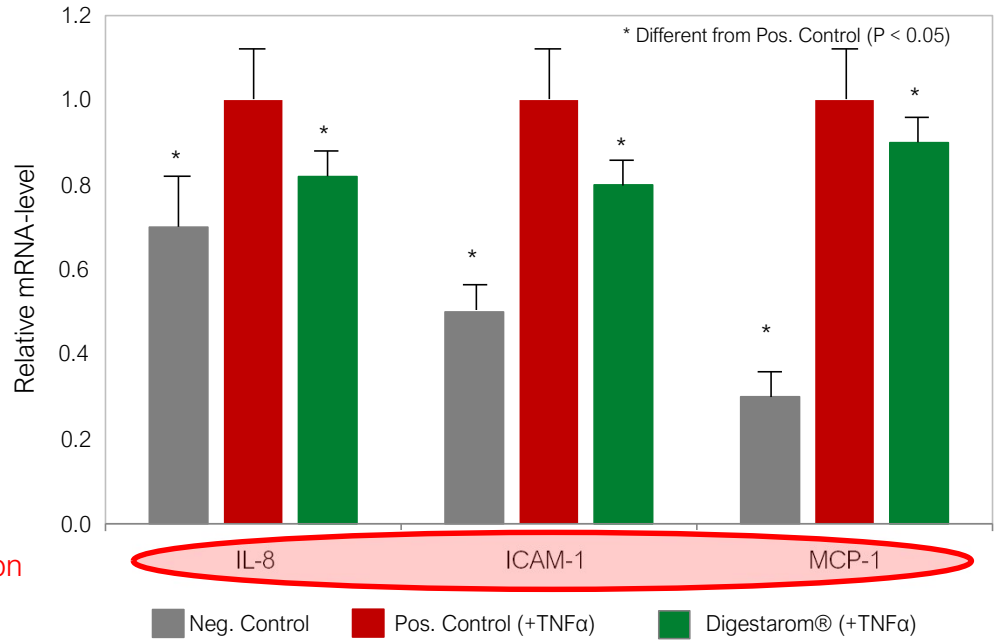
*Milder but persistent response of the immune system

More nutrients and more energy required when subclinical inflammation is present

Reducing expression of genes related to NF-κB (inflammatory response) with Digestarom®

In vitro test : Caco-2

1. without TNFα (Negative control)
2. with 10 ng/ml of TNFα (Positive control)
3. with 10 ng/ml of TNFα + Digestarom®

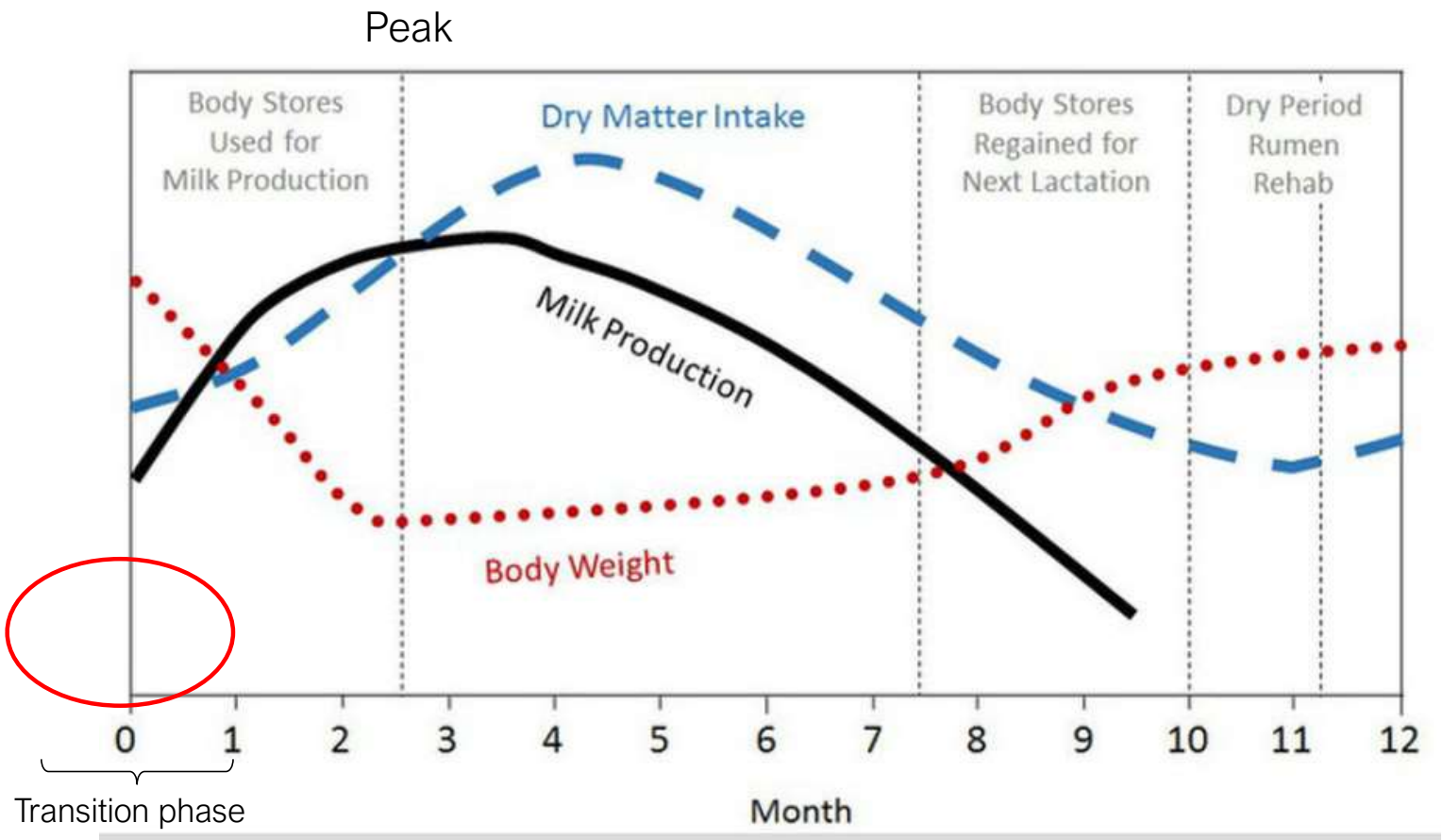


Inflammation markers

Reducing inflammation

Source: University of Gießen, *unpublished data*

Following through the challenges



Factors influencing colostrum quality

Detrimental factors

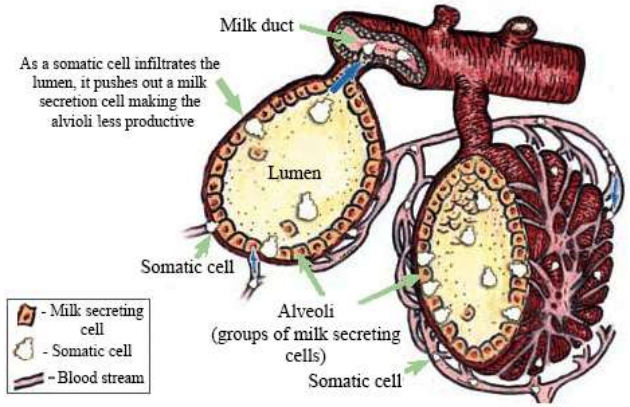
- Short dry period
- Inadequate nutrition
- Environmental stress
- Pen stress

Positive factors

- Age of dam
- Vaccinations
- Bolster stress coping capacity?

	Lactation				
Item	1	2	3	4+	SEM
n[2]	172	130	94	93	
IgG, mg/mL	83.5[b]	92.9[b]	107.4[a]	113.3[a]	3.8
IgG, g	532.8[c]	579.0[bc]	619.6[ab]	690.2[a]	28.2
Volume,[3] L	6.2	6.1	6.8	6.5	0.5
Calving ease[4]	1.6	1.7	1.6	2.1	0.2

Kehoe et al. 2011



Experimental design

Commercial trial evaluating influence of Digestarom® Dairy on colostrum quality

Location: Commercial dairy, ~ 2600 cows, California, USA
Design: 2 replicates per treatment, on/off design, no overlap of treatments
Animals: Close-up groups fed 30 day pre-calving, multiparous only

- Control, n = 530 cows, basal dry cow ration
- Treatment, n = 457 cows, basal dry cow ration, 2 g/cow/d Digestarom® Dairy

Data:

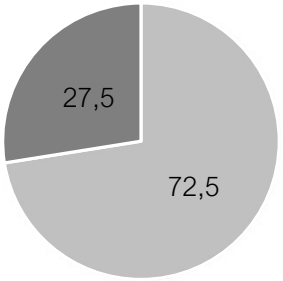
- Brix % value recorded on 1st milking colostrum
  - Indicator of IgG content, where >21% Brix = „Good quality“
- Colostrum yield recorded
- IgG concentration (mg/ml) = 6.0052 x Brix % - 49.292
- Total IgG (g) calculated using colostrum density estimate, 1.043 kg/m^3

Statistical analyses: GLIMMIX procedure of SAS (Cary, NC, USA)

- Values in figures reported as least square means

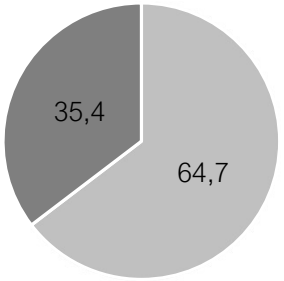
Digestarom® Dairy enhances herd colostrum quality

Control 2nd Parity



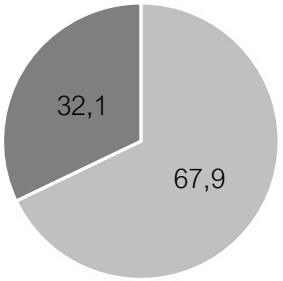
≤21% Brix ■ >21% Brix

Control 3rd+ Parity



≤21% Brix ■ >21% Brix

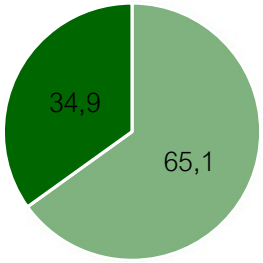
Control Overall



≤21% Brix ■ >21% Brix

Digestarom® Dairy 2nd Parity

+7.4% pts

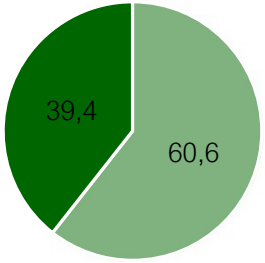


■ ≤21% Brix ■ >21% Brix

P < 0.10

Digestarom® Dairy 3rd+ Parity

+4.0% pts

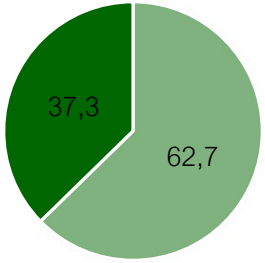


■ ≤21% Brix ■ >21% Brix

P > 0.10

Digestarom® Dairy Overall

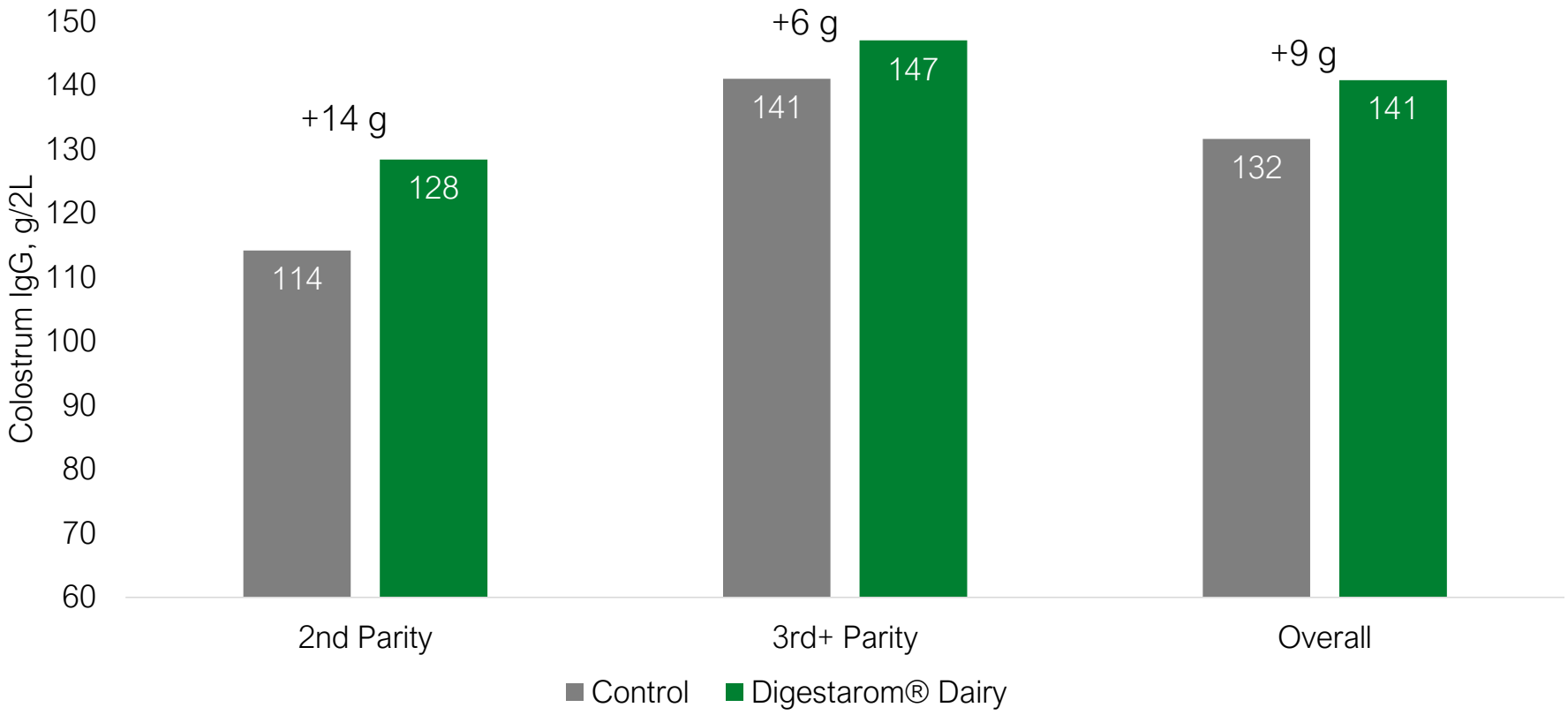
+5.2% pts



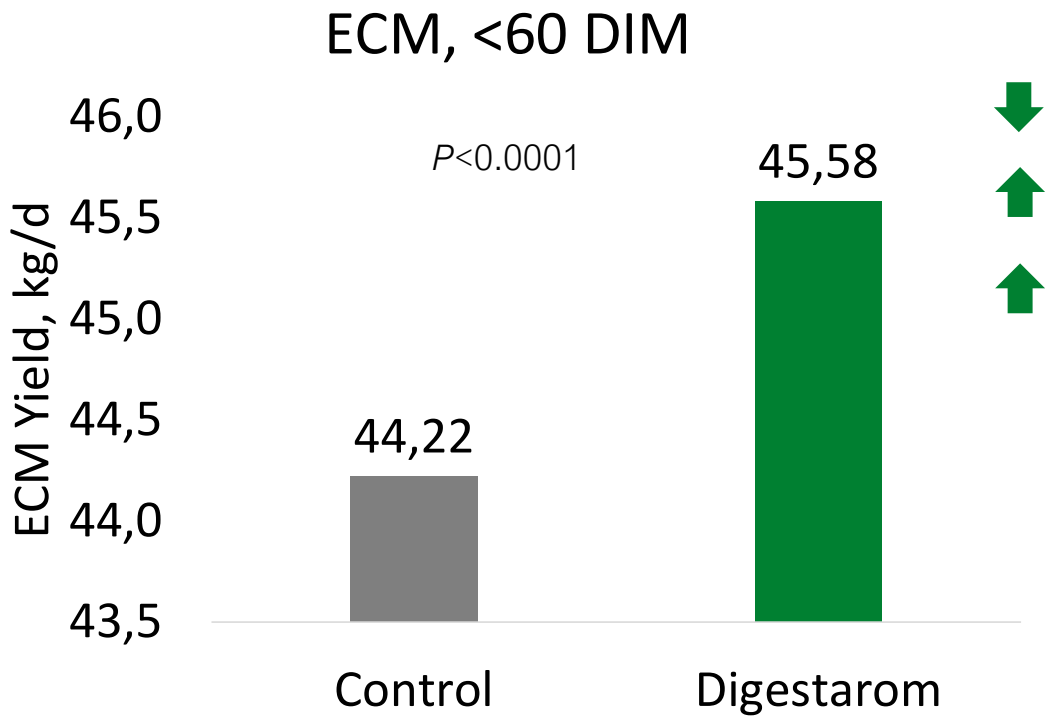
■ ≤21% Brix ■ >21% Brix

P < 0.10

Digestarom® Dairy delivers more IgG/L



Transition success with Digestarom® Dairy



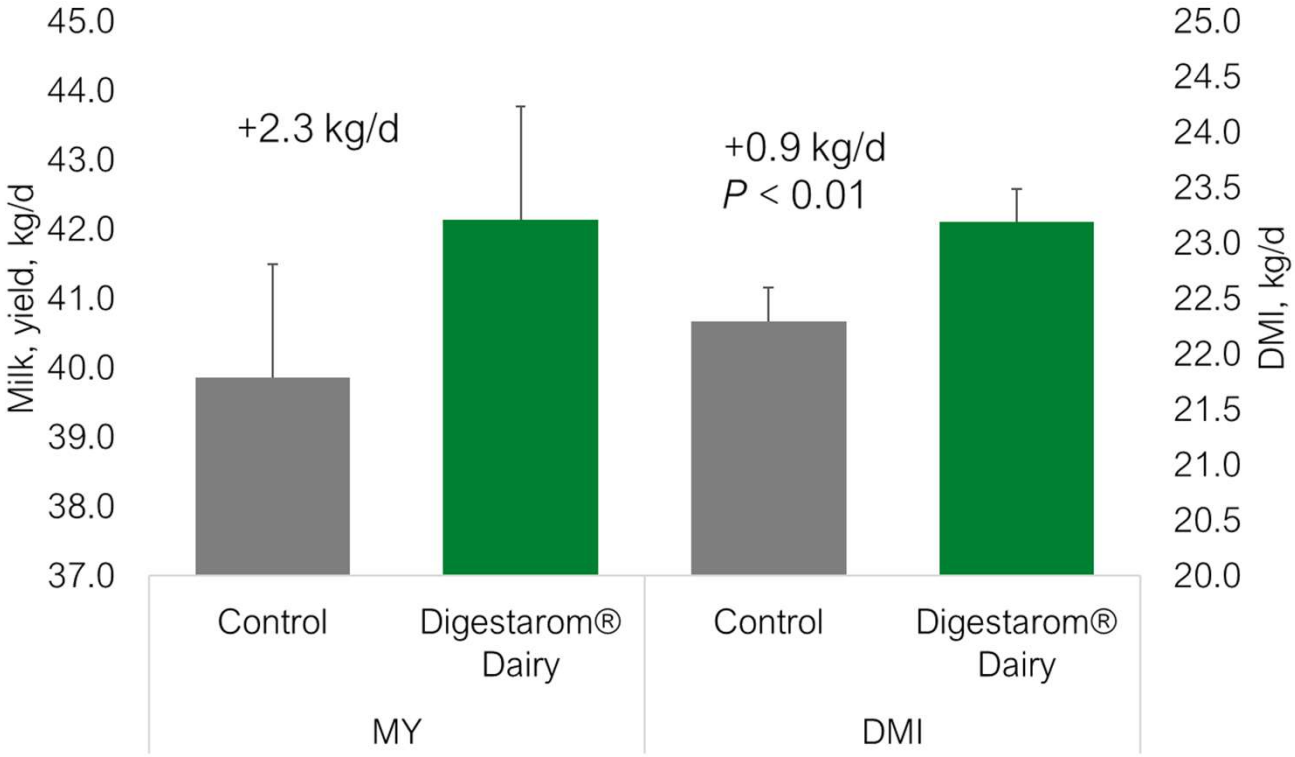
- Inflammation
- Glucose supply
- Milk yield

Baumgard 2017

D.Dairy.002
Sci & Solutions, V65

Transition success with Digestarom® Dairy

Fresh cow <30 day, n=75



„During transition, circulating **glucose is prioritized** to the non-insulin-dependent glucose transporters, which are only expressed on **immune cells** and the **mammary gland**“ Lopreiato et al. 2020

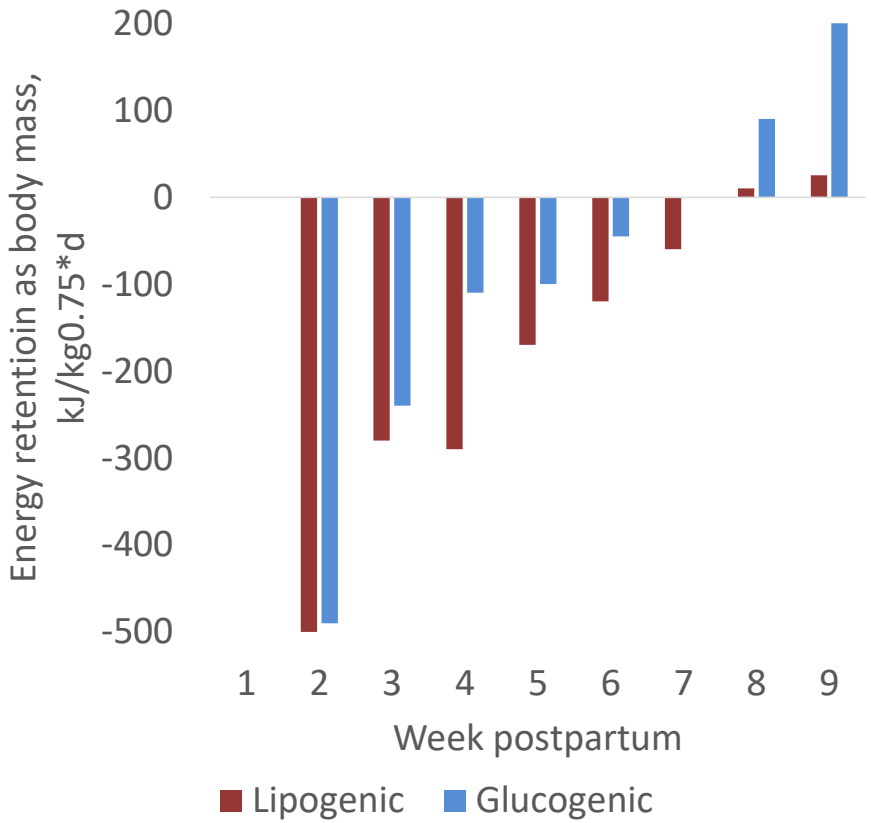
Link between glucose and fertility

Glucogenic diet resulted in:

- Earlier resolving of NEB
- Progesterone detection 6 days earlier

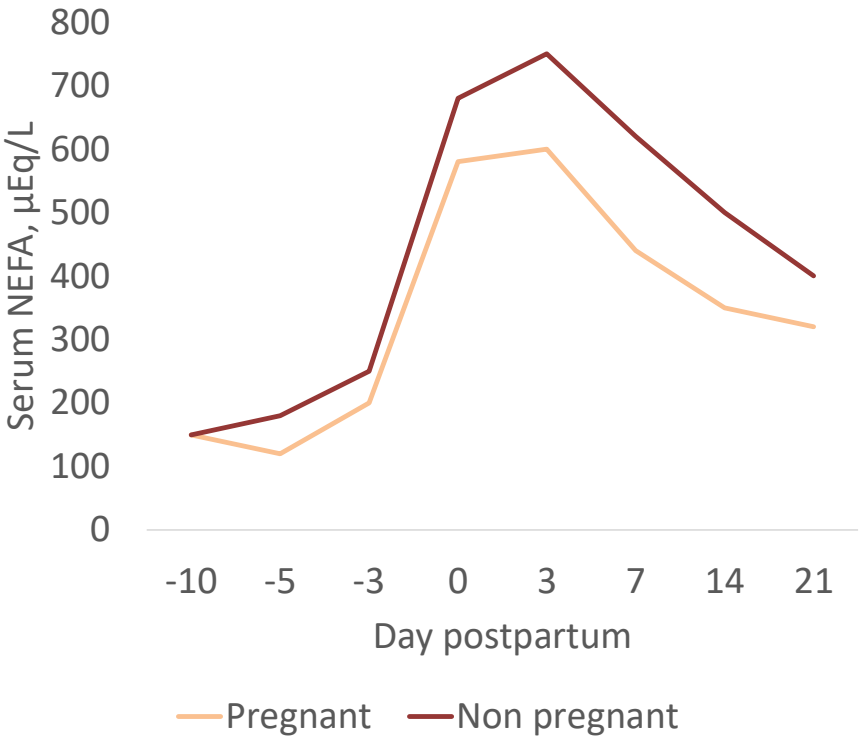
Manage the inflammation ➡

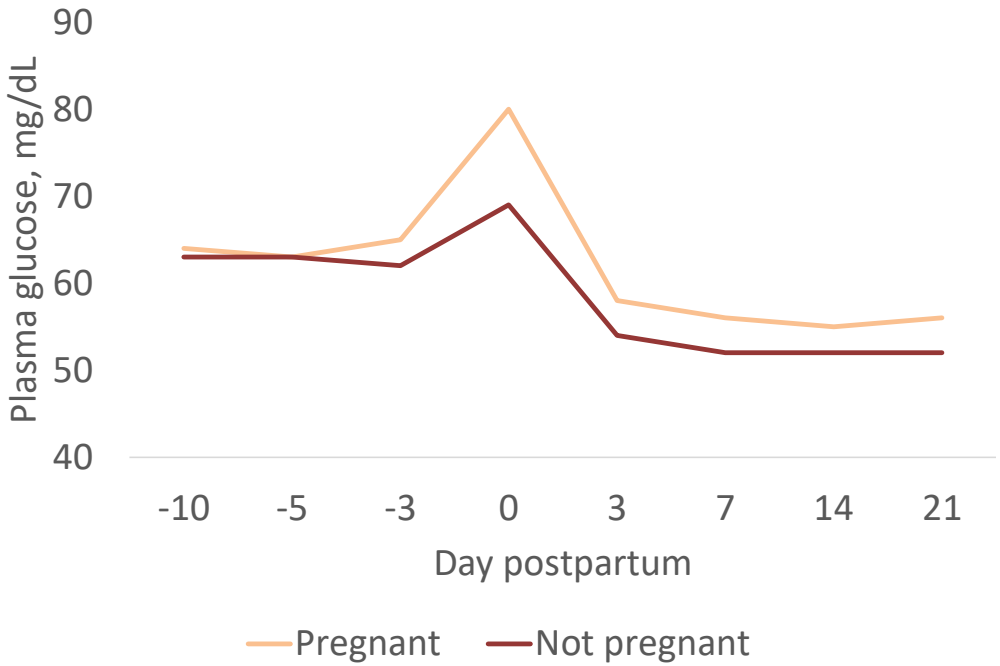
➡ direct the energy flow



Knegsel 2007

Glucose as indicator of 1st AI success



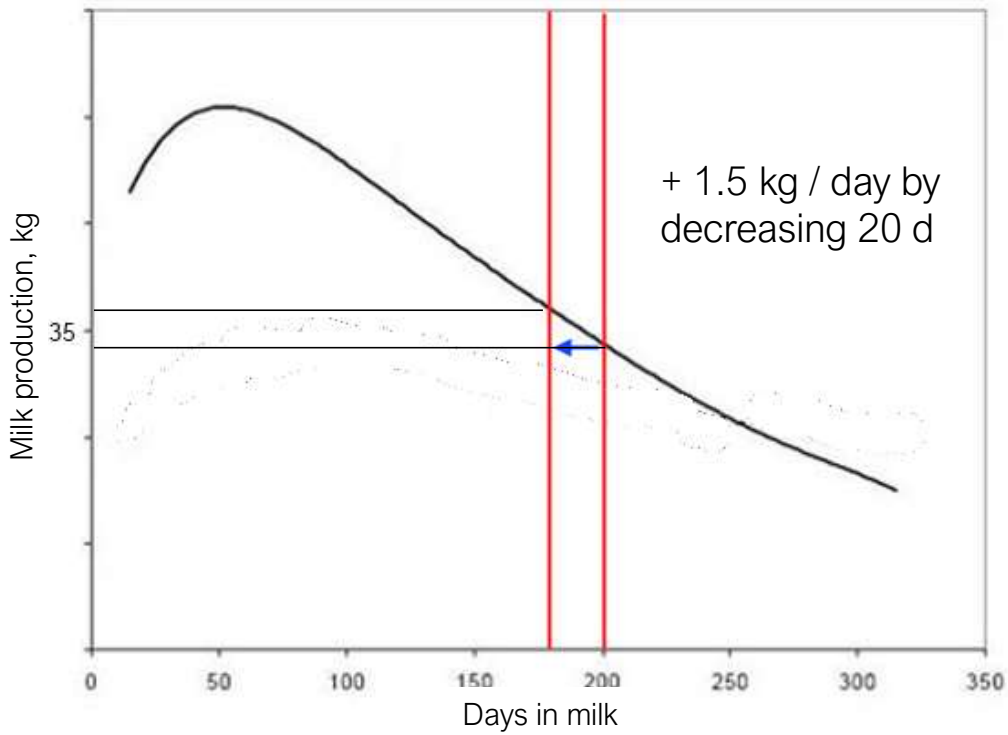


Garveric *et al*., 2013

Improving fertility has significant economic impact

Metabolic diseases delaying ovulation

Preventative nutrition to manage stress

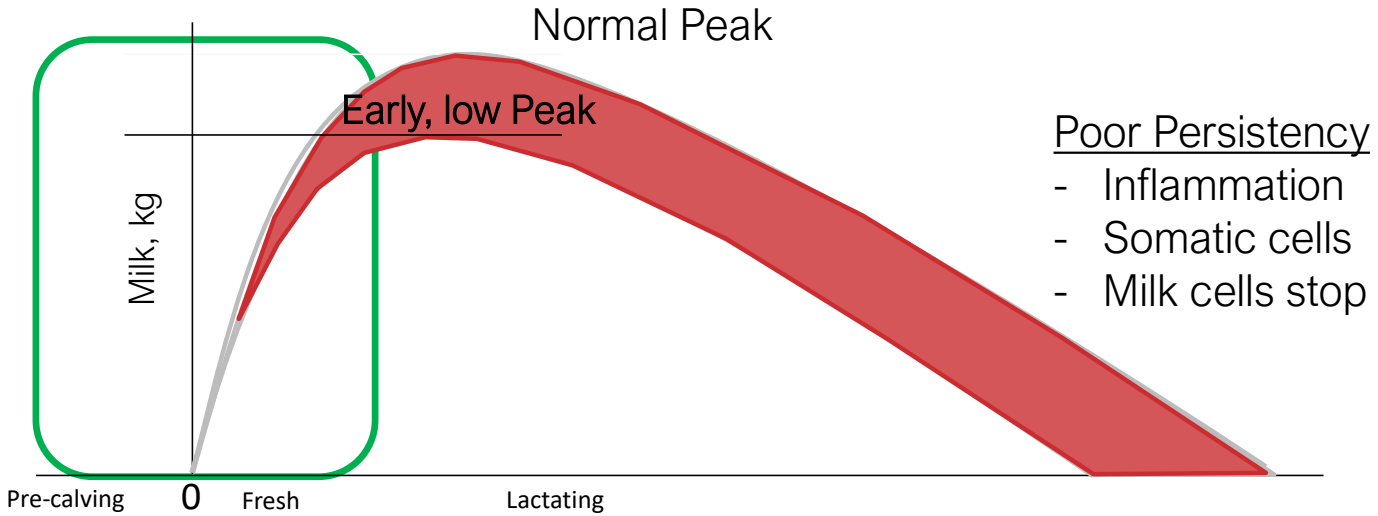


DHI, 2015

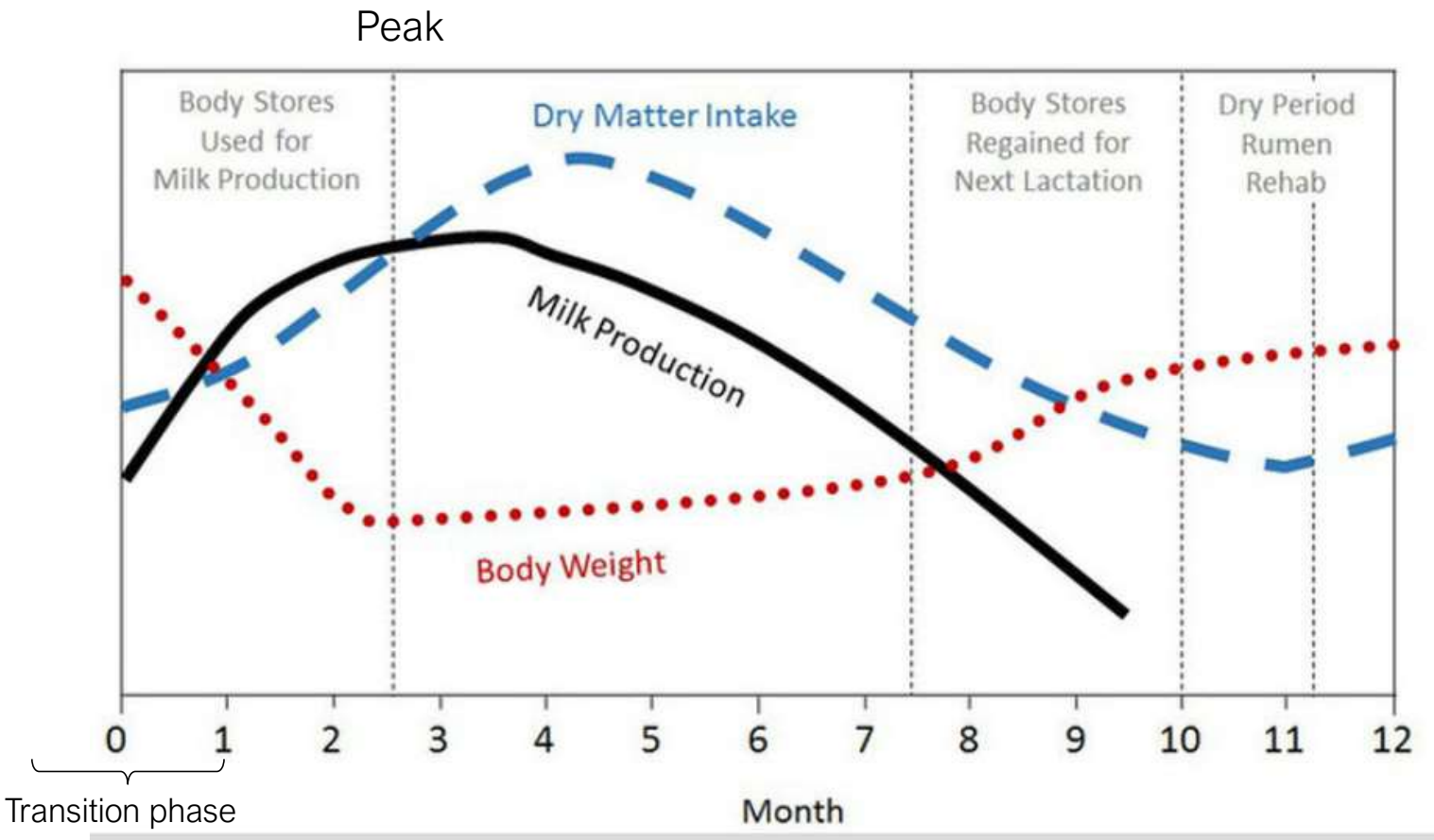
How phytogenic feed additives help throughout lactation

After peak, can only maintain persistancy

Healthy transition increases peak lactation



Sub-acute rumen acidosis can occur anytime

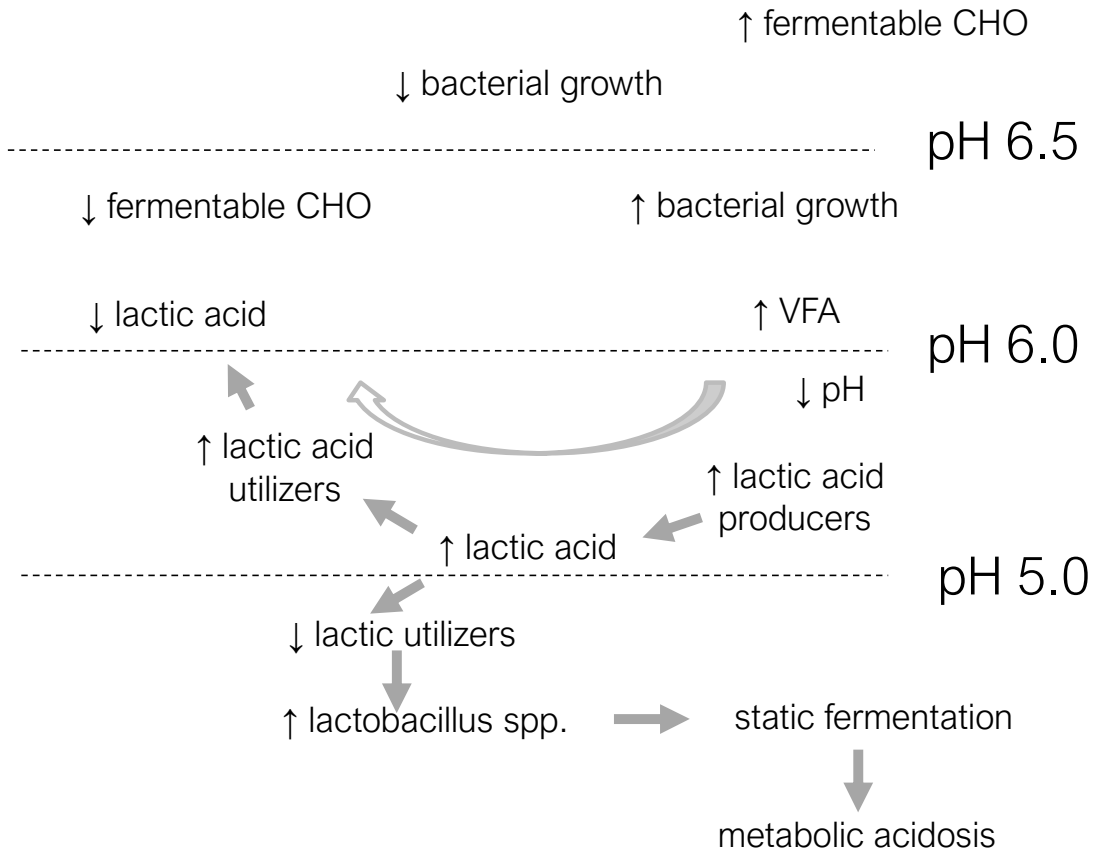


Performance starts with rumen stability

↑ Performance = ↑ Challenges

- More rapid CHO
- Less peNDF
- Easier sorting
- Fluctuating intake
- Metabolic disorders

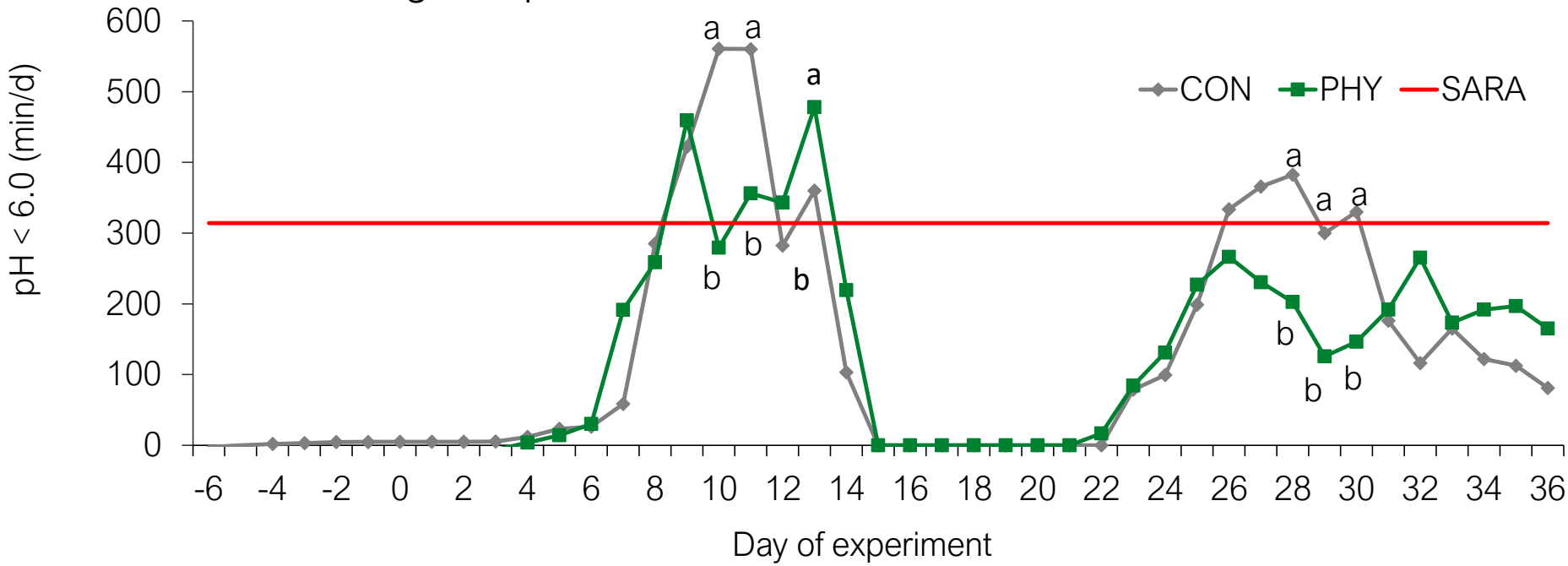
Perpetual SARA risk



McAllister et al.1996

Digestrom® Dairy reduces SARA duration

Effect of SARA challenge on pH < 6.0



P < 0.05

pH < 6.0 for 314 min/d
(Zebeli *et al.*, 2008; Neubauer *et al.*, 2017)

Kröger *et al.*, 2017

Positive influence on rumen motility

Higher performance = rumen challenge:

- More NFC
- High SARA risk
- Potential rumen pH rollercoaster
- Inflammation catalysts

Parameter	Digestarom®	Levabon®
Feed bunk		
Intake, DM	↑ 7%	↑ **20%**
Eating time	↑ 15%	↑ **31%**
Chewing activity		
Total chewing (min/day)	↑ 33 %	↑ 31%
Ruminating		
Ruminating (min/day)	↑ **55 %**	↑ 30%
Boli (Number/day)	↑ **50 %**	↑ 25%
Ruminal pH		
Time of pH < 6.0 (min)	↑	↑

Kröger et al. 2017

Digestarom® Dairy modulates microbiota

- Rumen microbiota highly diverse
  - Substrate flexibility
  - Robust recovery
  - Little understood
- SARA shifts to low pH thriving spp.
  - Gram –ve amyolytic
- Digestarom® Dairy maintains diversity
  - Faster recovery
  - More rumen efficient
  - Stimulate butyrate producing spp.
    - Enhance VFA absorption

OTU Number	Taxonomic classificaiton according to SILVA and NCBI best BLASTn hit ≥ 97% identity	Identity	Phase 1	
			CON	Digestarom® Dairy
OTU_6	*Ruminococcaceae* (f)	12		**
OTU_14	*Syntrophococcus* (g)	100		**
OTU_17	*Ruminococcaceae* (f)	97		**
OTU_21	*Prevotella ruminicola* (s)	100		**
OTU_23	*Ruminococcus flavefaciens* (s)	99		
OTU_26	*Saccharofermentants* (g)	100		
OTU_31	*Ruminococcaceae* (f)	97		**
OTU_48	*Ruminococcus* (g)	97		
OTU_50	*Prevotella ruminicola* (s)	99		**
OTU_52	*Roseburia intestinalis* (s)	97		
OTU_59	*Shuttleworthia* (g)	97		**
OTU_60	*Ruminococcaceae* (f)	97		**
OTU_76	*Prevotella* (g)	99		**
OTU_95	*Moryella indoligenes* (s)	96		**
OTU_99	*Olsenella* (g)	99		**
OTU_100	*Eubacterium pyrubativorans* (s)	99		**

Relative abundance %
≥ 2.0
1.0 - 2.0
0.5 - 1.0
0.2 - 0.5
< 0.2
** $P < 0.01$

** $P<0.01$

Neubauer *et al.*, 2018

Modulating inflammatory biogenic amines

Effects of Digestarom® Dairy on rumen biogenic amines

Histamine

- Acute inflammatory response
- Role in the pathology of laminitis

Methylamine

- Degraded toxic metabolites and absorbed

Spermidine, Spermine

- Cause oxidative stress

Biogenic amines	CON	Dig-Dairy
Methylamine	43.7	36.5 ↓
Isopropylamine	20.7	14.9
Pyrrolidine	16.7	10.3 ↓
Putrescine	37.5	29.9
Histamine	21.6	7.42 ↓↓
Tyramine	2.00	1.21
Spermindine	19.8	13.9 ↓
Spermine	1.97	0.902 ↓

Adapted from Humer et al. 2018

Digestarom® Dairy modulates VFA

Benefits of more neutral pH

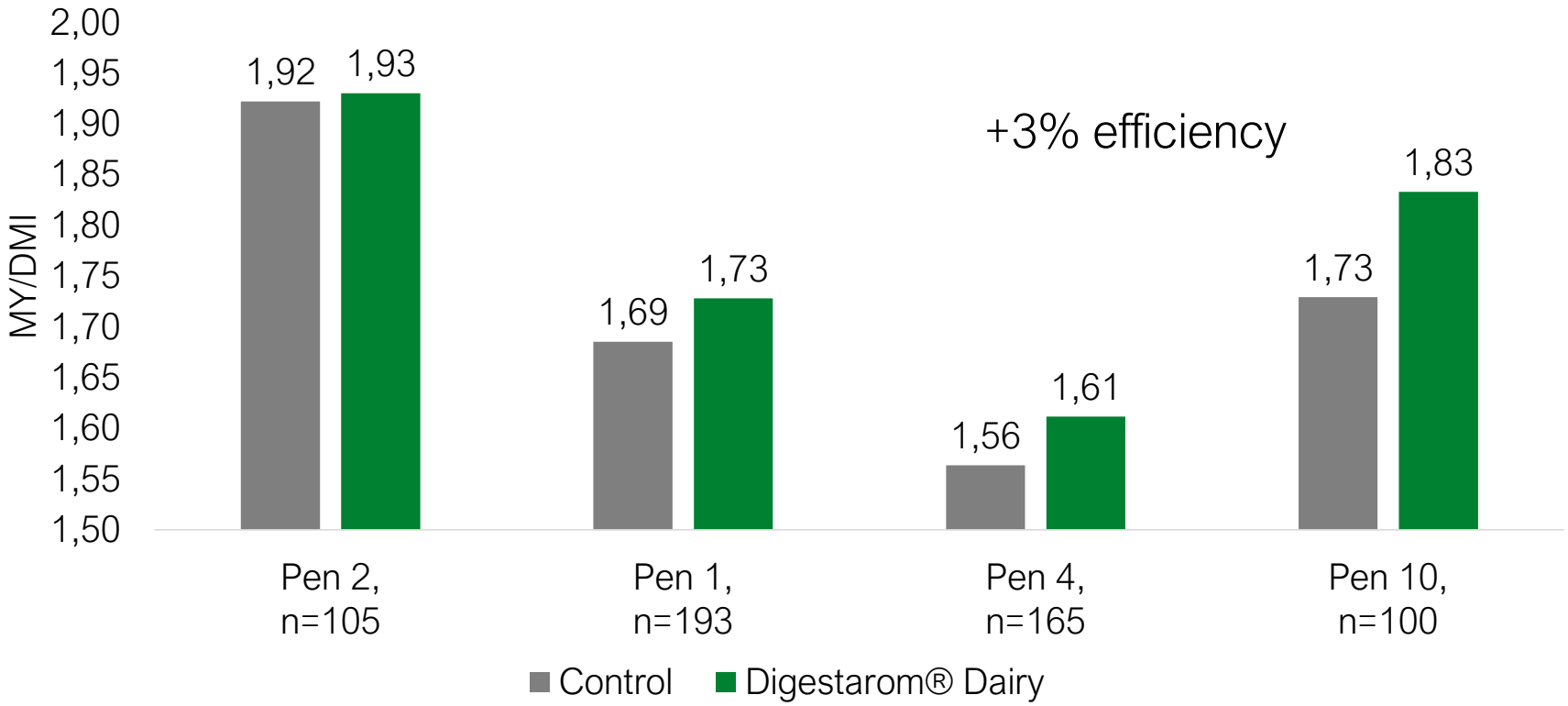
- More microbial synthesis
  - ↑ NH_3-N capture
  - ↑ VFA production
  - Higher A+B/P
- Protozoa maintained
  - ↑ Archaea (CH_4, fiber digestion)
  - Starch digestion slowed

	Control	Digestarom® Dairy
Total SCFA, mM	118.8	124.4
% total SCFA		
Acetate	54.7	55.9
Propionate	23.8	21
Butyrate	13	16
A+B/P	2.8	3.4

23% more butyrate
36% less lactic acid
34% less NH_3-N

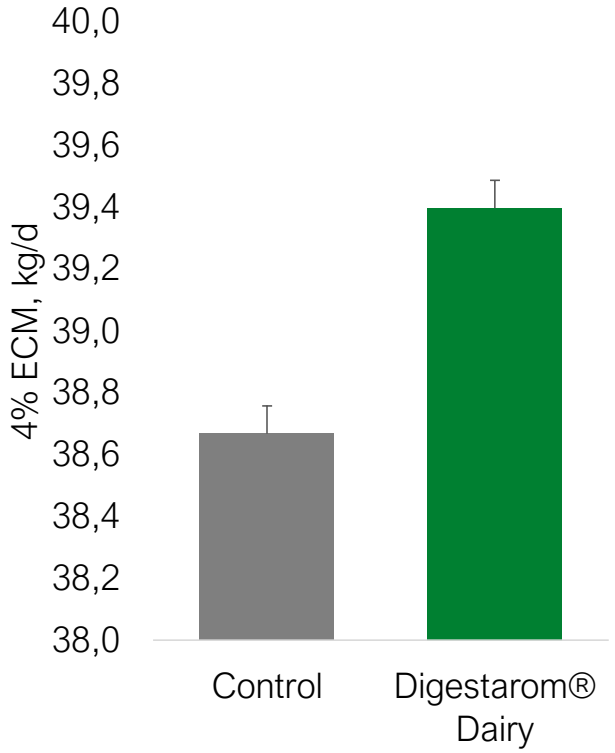
Neubauer *et al.*, 2018

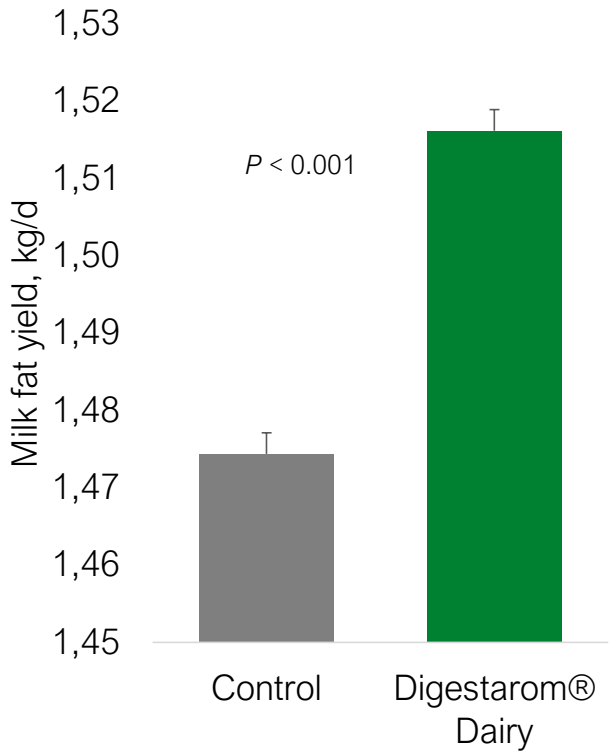
Improving efficiency and income over feed cost

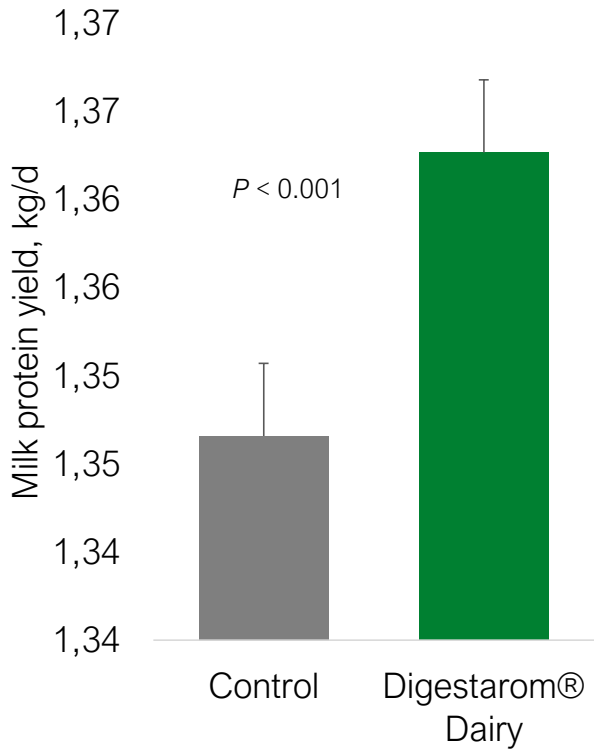


Herd Performance – Processor report

n=~900 lactating cows

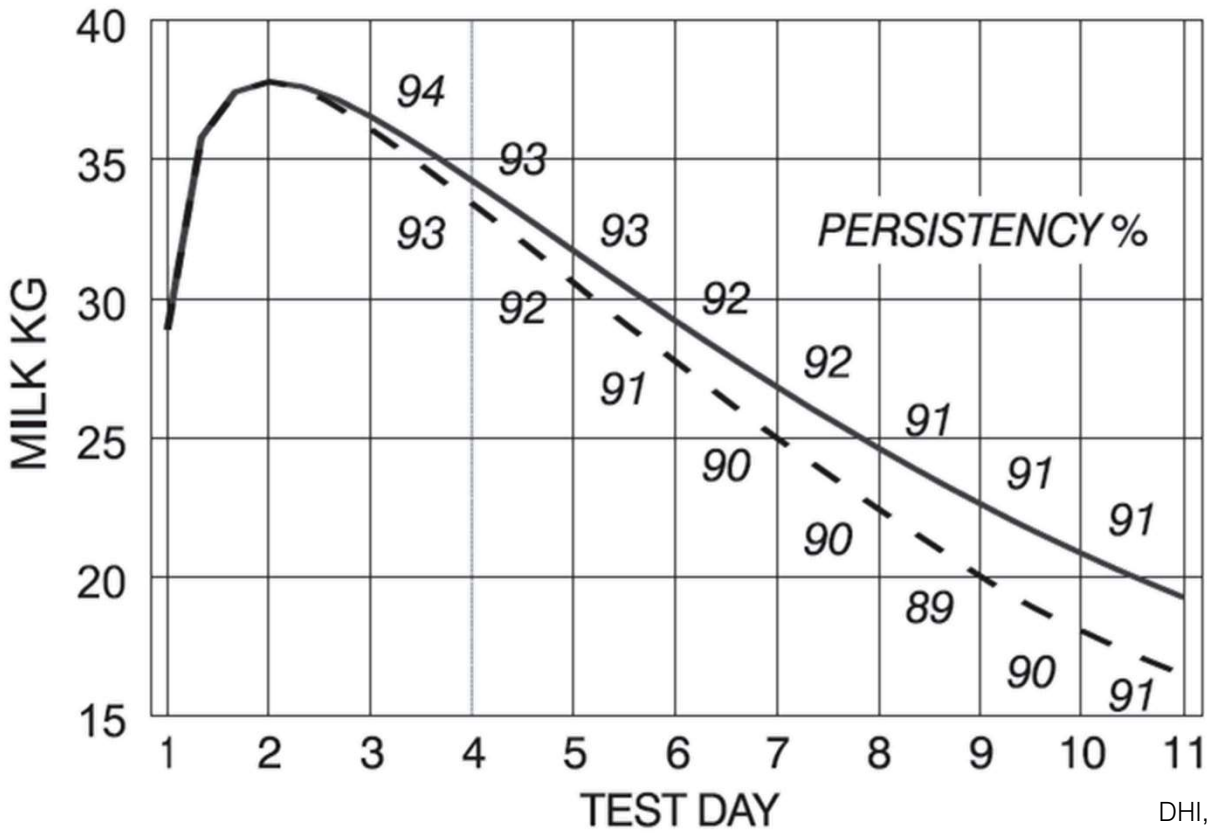






Why persistency a benchmark for herd health

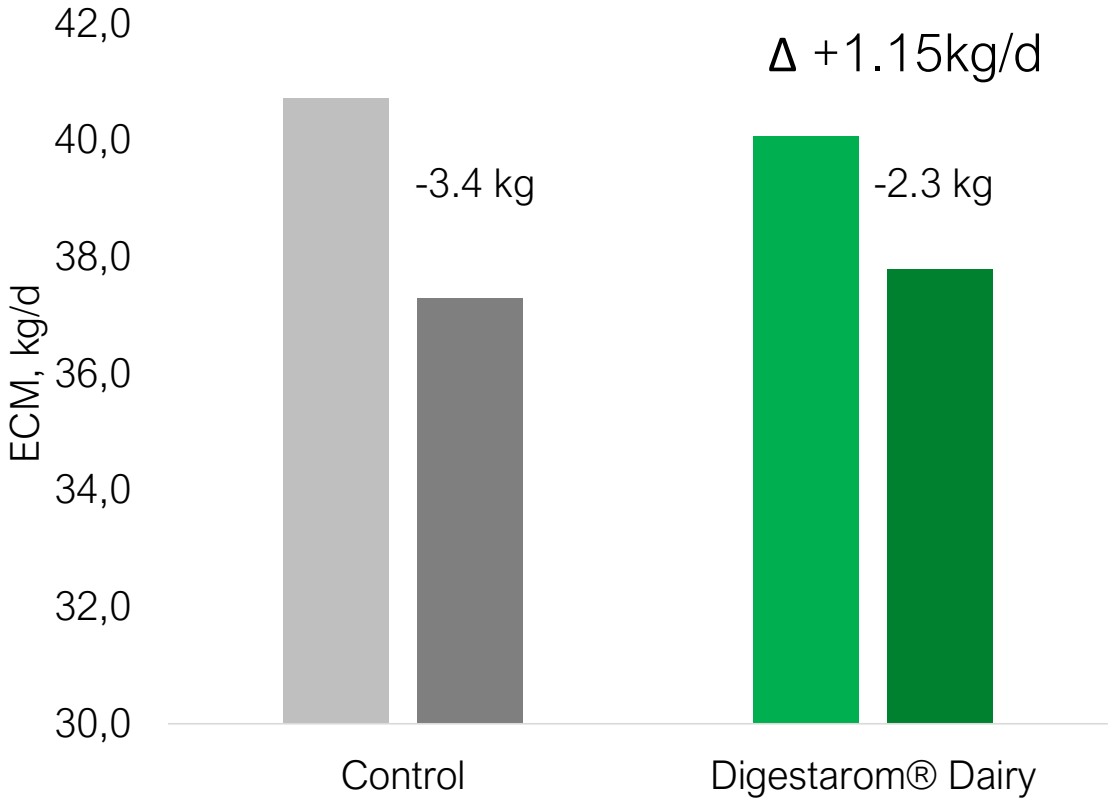
- Stress challenges shorten lactation
  - Nutrition
  - Environment
  - Group changes
- Glucose supply limiting
- Secretion cells „shut down“
  - No reactivation
- Increase risk of high BCS
  - Group uniformity



DHI, 2015

Digestarom® Dairy enhances persistency, mitigates stress challenges

8 week data



Implications

Digestarom® Dairy has positive effects throughout lactation:

- More ECM, from yield and solids, enhances returns
- 3% improved feed efficiency, better IOFC
- Positive influence on fresh cow DMI and milk production
- Managing stressors holding back production potential
  - Inflammatory stress
  - Oxidative stress
- Higher quality colostrum

Questions and Discussion