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IN KRAKOW

Dairy Cows in Questions

"Immunosuppression and hypocalcemia in the transition period of dairy cows – how can we prevent them?"

How can we support the immune response of a cow by the diet?

Can We Feed Dairy Cows to Improve Transition Immunity?

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Pennsylvania State University

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Presentation Outline

- Transition Cow Immune Challenges
- Inflammation, Nutrition, Homeorhesis
- Mounting an Immune Response
- Supporting an Activated Response
- Summary Recommendations

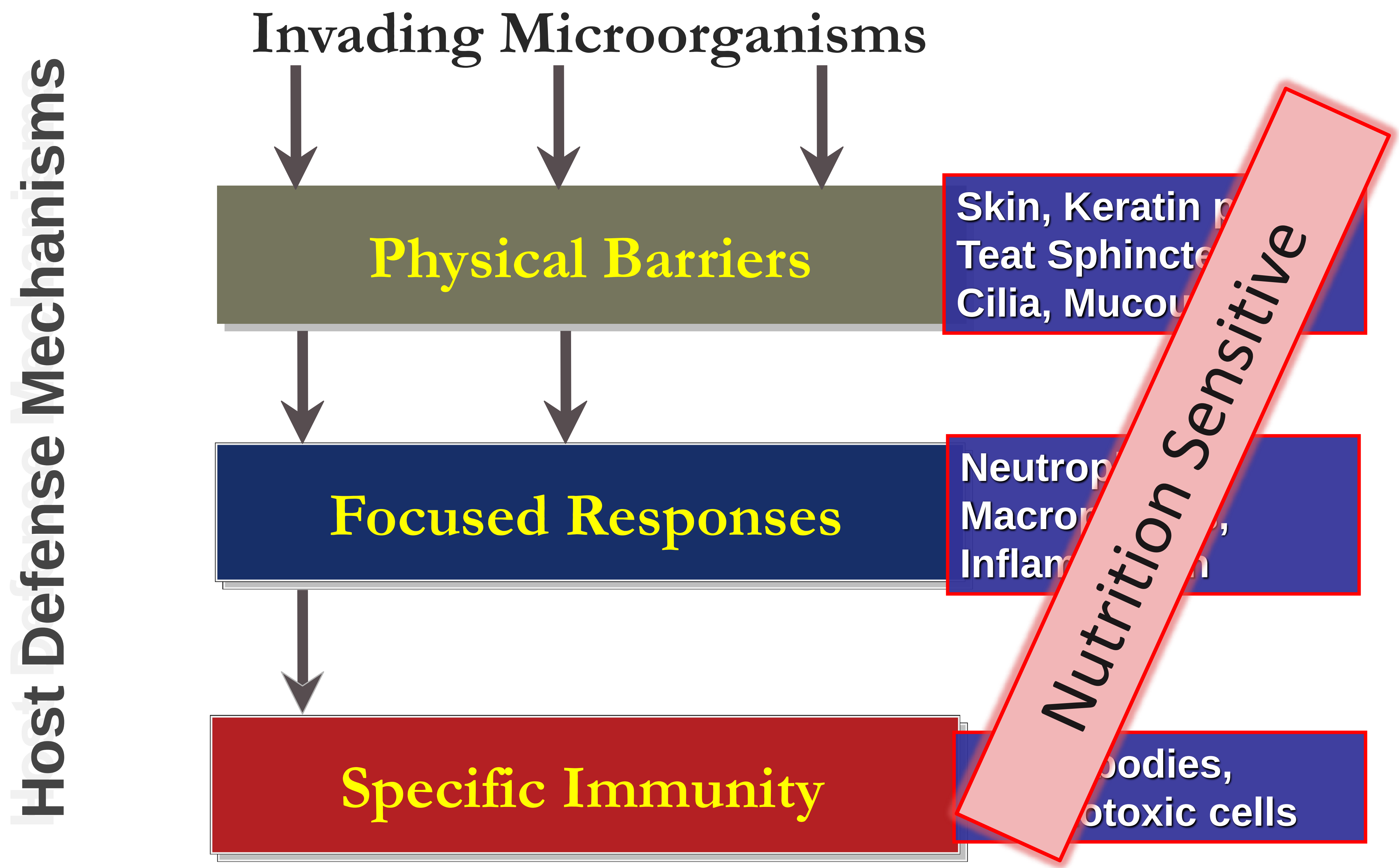
Transition Period Goals

- Low prevalence of postparturient diseases
- Maintain immunocompetency to pathogens
- Minimize loss (<0.5 units) or maintain BCS postpartum

- Support efficient milk production
- Control/decrease days to first ovulation and maintain/enhance fertility
- Low stillborn rate and healthy calves

Nutritional Control Points in Managing Transition Success

- Minimize drop in prepartum DMI
  - Improved postpartum DMI
  - Minimize increase in NEFA
- Adequate dietary supply of ME and MP
  - Based on observed intake and accounts for variation
- Minimize risk of hypocalcemia
  - Hypocalcemia gateway disease
- Maintain a competent immune response
- Support and control the inflammatory/immune response


Host Defense Mechanisms
Invading Microorganisms
Physical Barriers
Focused Responses
Specific Immunity
Skin, Keratin p
Teat Sphincte
Cilia, Mucou
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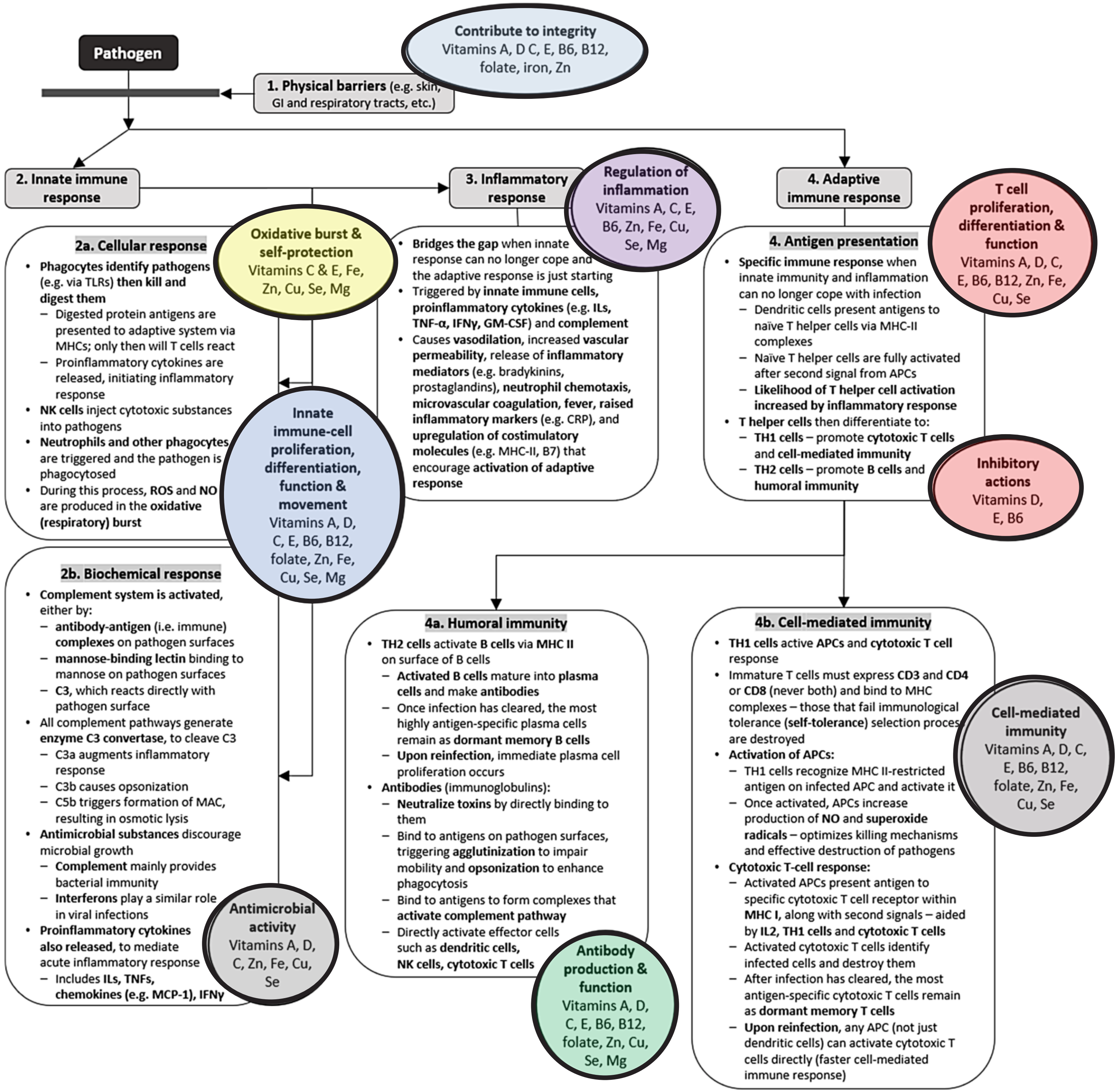
Nutrition Sensitive



Nutritional Impacts on Immunity

Macronutrients: energy, amino acids, fatty acids

Micronutrients: trace minerals and vitamins all can influence many aspects of immune response.

A Review of Micronutrients and the Immune System–Working in Harmony to Reduce the Risk of Infection

Nutrients **2020**, *12*(1), 236; https://doi.org/10.3390/nu12010236

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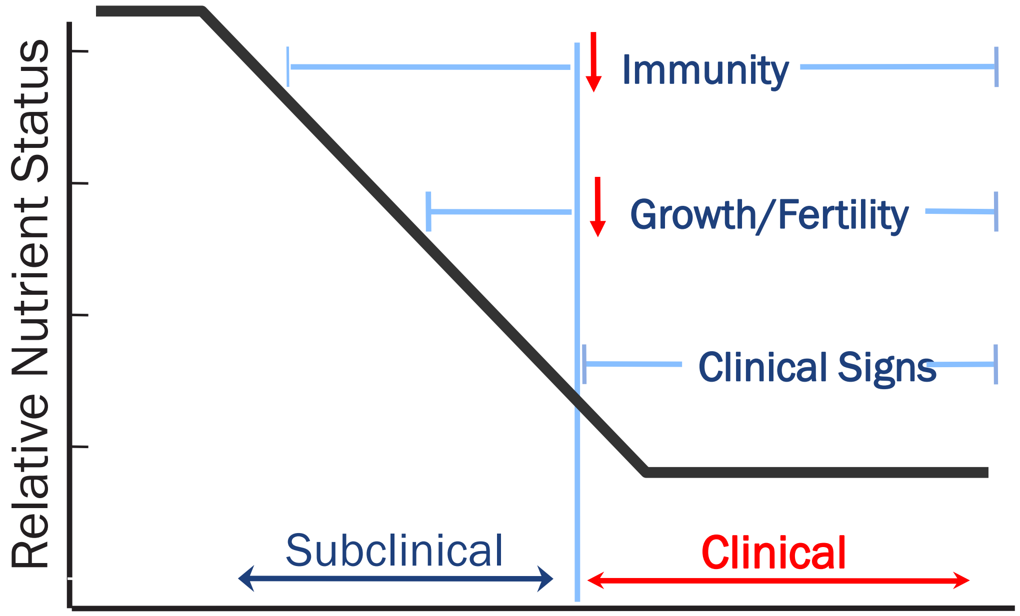
Dichotomy of Nutrition-Immunity Interaction

- Homeorhesis Impacts – immune response is of “low priority” in the face of nutritional deficits



- Immune Activation – inflammatory response initiates immune responses thus requiring high nutrient input to support
  - Energy – Glucose utilization
  - Amino acids – Acute phase proteins, cytokines, peptides, etc
  - Antioxidant nutrients
  - Immune modulatory nutrients – Ca, P, Se, Cu, Vitamins A, D, E

Wikse, 1992 TAMV Beef Cattle Short Course

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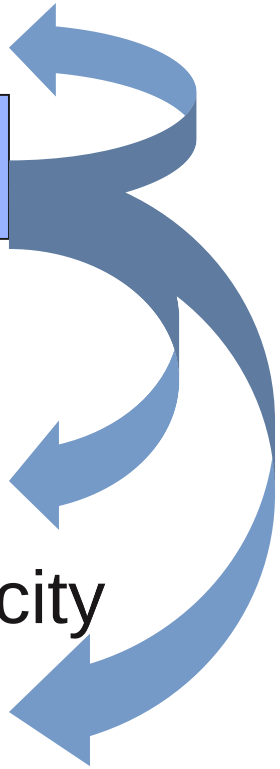
Transition Homeorhetic Nutrient Prioritization

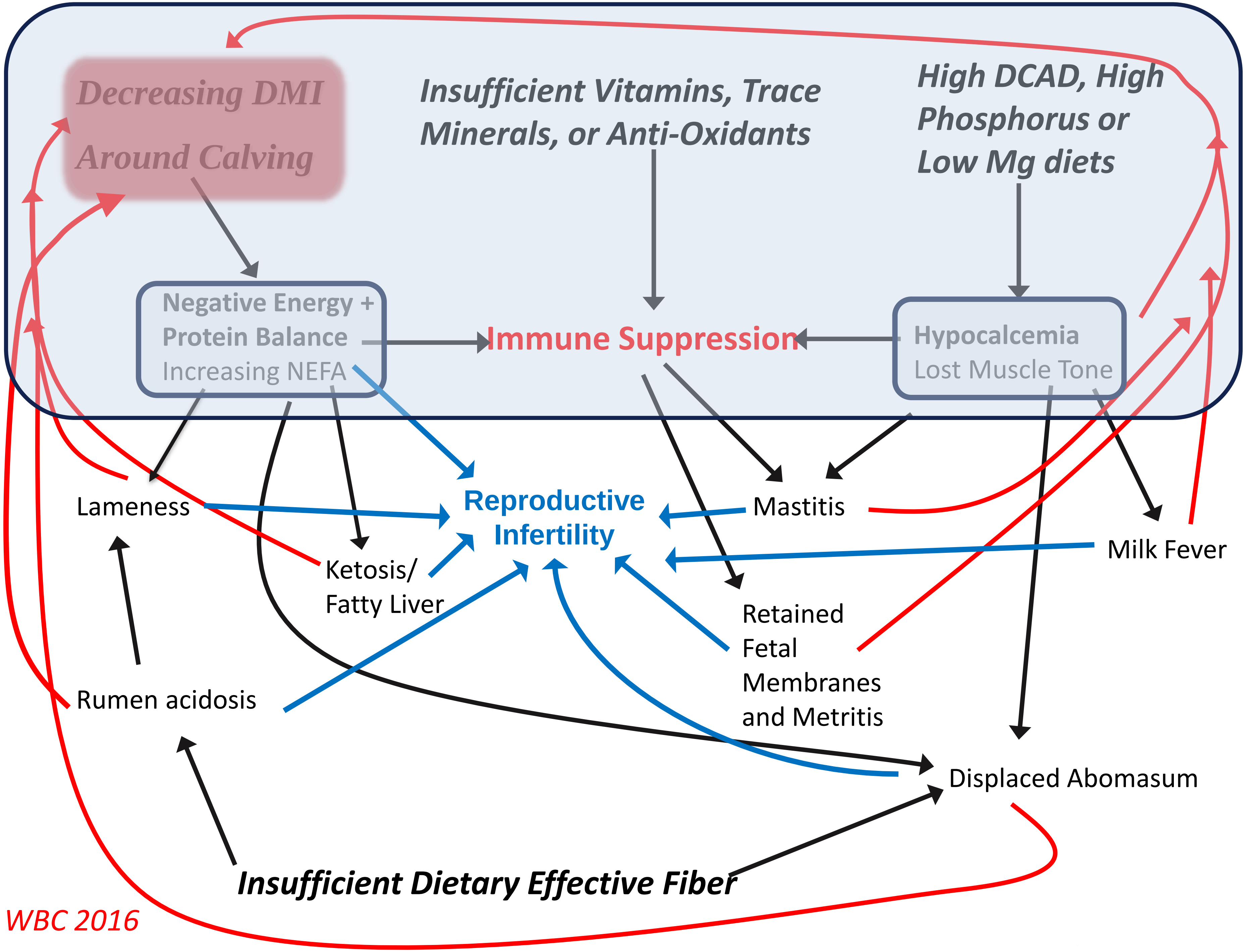
Prepartum

- Maintenance
- Pregnancy
- Frame growth
- Lactation
- Reserves
- Reproductive cyclicity
- Immune response



- High priority to maintain established pregnancy
- Body reserves (adipose, minerals, vitamins) will be mobilized if not sufficiently supplied in the diet
- Maternal protein mobilized to meet fetal needs
- Immune response compromised due to inadequate nutrients to establish a response

Adapted from Bauman and Currie, J Dairy Sci 1980



Adapted from Goff, WBC 2016

Antioxidant Nutrients

Oxidative stress is an important factor contributing to dysfunctional inflammatory responses in metabolically stressed cows. During the transition period there is potential for imbalances in expression of reactive oxygen species and availability of antioxidants.

Nutrient	Active Component	Function
Vitamin A	β-Carotene	Prevents fatty acid peroxidation chain reactions
Vitamin C	Ascorbic Acid	Radical scavenger
Vitamin E	α-Tocopherol	Disrupts fatty acid peroxidation chain reactions
Selenium	Thioredoxin reductase	Redox signaling and reduces reactive oxygen species (ROS)
Selenium	Glutathione peroxidase	Redox signaling and reduces reactive oxygen species (ROS)
Copper	Ceruloplasmin	Oxidase activity; Peroxyl radical scavenger
Copper-Zinc	Superoxide dismutase	Converts cytosol superoxide to H_2O_2
Zinc	Metallothionein	Cysteine rich radical scavenger
Manganese	Superoxide dismutase	Converts mitochondrial superoxide to H_2O_2
Iron	Catalase	Converts H_2O_2 to water

Sordillo and Aitken Vet Immunol Immunopathol 2009; Sordillo JDS 2016

J. Dairy Sci. 95:4568–4577
http://dx.doi.org/10.3168/jds.2012-5404

Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or methionine hydroxy analogue chelates

L. M. Nemec,* J. D. Richards,† C. A. Atwell,† D. E. Diaz,† G. I. Zanton,† and T. F. Gressley*[1]
*Department of Animal and Food Science, University of Delaware, Newark 19716
†Novus International Inc., St. Charles, MO 63304

The effect of injectable trace minerals (selenium, copper, zinc, and manganese) on peripheral blood leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows

CrossMark

V.S. Machado [a], G. Oikonomou [a], S.F. Lima [a], M.L.S. Bicalho [a], C. Kacar [c], C. Foditsch [a], M.J. Felippe [b], R.O. Gilbert [b], R.C. Bicalho [a,*]

J. Dairy Sci. 97:3728–3738
http://dx.doi.org/10.3168/jds.2013-7331

Effects of hydroxy trace minerals on oxidative metabolism, cytological endometritis, and performance of transition dairy cows

T. Yasui,* C. M. Ryan,* R. O. Gilbert,† K. R. Perryman,‡ and T. R. Overton*[1]

Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows

CrossMark

V.S. Machado [a], M.L.S. Bicalho [a], R.V. Pereira [a], L.S. Caixeta [a], W.A. Knauer [a], G. Oikonomou [a], R.O. Gilbert [b], R.C. Bicalho [a,*]

J. Dairy Sci. 99:1–16
http://dx.doi.org/10.3168/jds.2015-10040

Supplementing Zn, Mn, and Cu from amino acid complexes and Co from Co glucoheptonate during the peripartal period benefits postpartal cow performance and blood neutrophil function

J. S. Osorio,*† E. Trevisi,‡ C. Li,§ J. K. Drackley,† M. T. Socha,# and J. J. Loor*†[1]

Metal ions in macrophage antimicrobial pathways: emerging roles for zinc and copper

Sian L. STAFFORD*†[1], Nilesh J. BOKIL†‡[1], Maud E. S. ACHARD*†, Ronan KAPETANOVIC†‡, Mark A. SCHEMBRI*†, Alastair G. McEWAN*† and Matthew J. SWEET†‡[2]

J. Dairy Sci. 105
https://doi.org/10.3168/jds.2021-20624

Effect of injectable trace mineral supplementation on peripheral polymorphonuclear leukocyte function, antioxidant enzymes, health, and performance in dairy cows in semi-arid conditions

T. H. Silva,[1,2] I. Guimaraes,[1] P. R. Menta,[1] L. Fernandes,[1] D. Paiva,[1] T. L. Ribeiro,[1] M. L. Celestino,[1] A. Saran Netto,[2] M. A. Ballou,[1] and V. S. Machado[1*]

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Zinc and Mastitis

- Peer-reviewed study outcomes:
  - Negative studies (6+)
  - Positive studies (9+)
  - Many other tech reports
- Study differences:
  - Source of Zn
  - Supplementation rate
  - Dietary Zn status vs. NRC
  - Statistical methods for SCC comparisons

Summary of 12 studies showing a 33% decline in SCC

TABLE 6. Effect on milk composition and somatic cell count (SCC) of supplementing lactating dairy diets with zinc methionine (ZM) complex[a].

Trial	Fat		Protein		SCC	
	Control	ZM	Control	ZM	Control	ZM
	(%)				(×10³/mL)	
Washington[b]	3.62	3.67	3.27	3.20	231	136
Washington[b]	3.21	3.58	3.01	3.01	218	176
Colorado[c]	3.56	3.37	3.19	3.11	560	282
Great Britain[b]	3.59	3.64	3.08	3.08	497	390
Illinois[b]	3.40	3.30	3.10	3.00	243	228
New York[b]	—	—	—	—	242	115
Colorado[b]	3.60	3.55	3.18	3.22	250	195
Arkansas[b]	3.44	3.38	3.21	3.17	—	—
Israel[b]	2.62	2.62	3.13	3.07	410	333
Missouri[c]	3.50	3.50	3.00	3.10	228	46
Missouri[c]	3.70	3.50	3.10	3.10	131	46
Germany[c]	—	—	—	—	95	81
Georgia[c]	4.00	4.20	3.20	3.10	—	—

[a]12 trials; 13 comparisons.

[b]Treatment diet provided between 180 and 200 mg Zn/d per head from ZM complex.

[c]Treatment diet provided between 360 and 400 mg Zn/d per head from ZM complex.

Kellogg et al., PAS 2004

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J. Dairy Sci. 93:4239–4251
doi:10.3168/jds.2010-3058

Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: A meta-analysis

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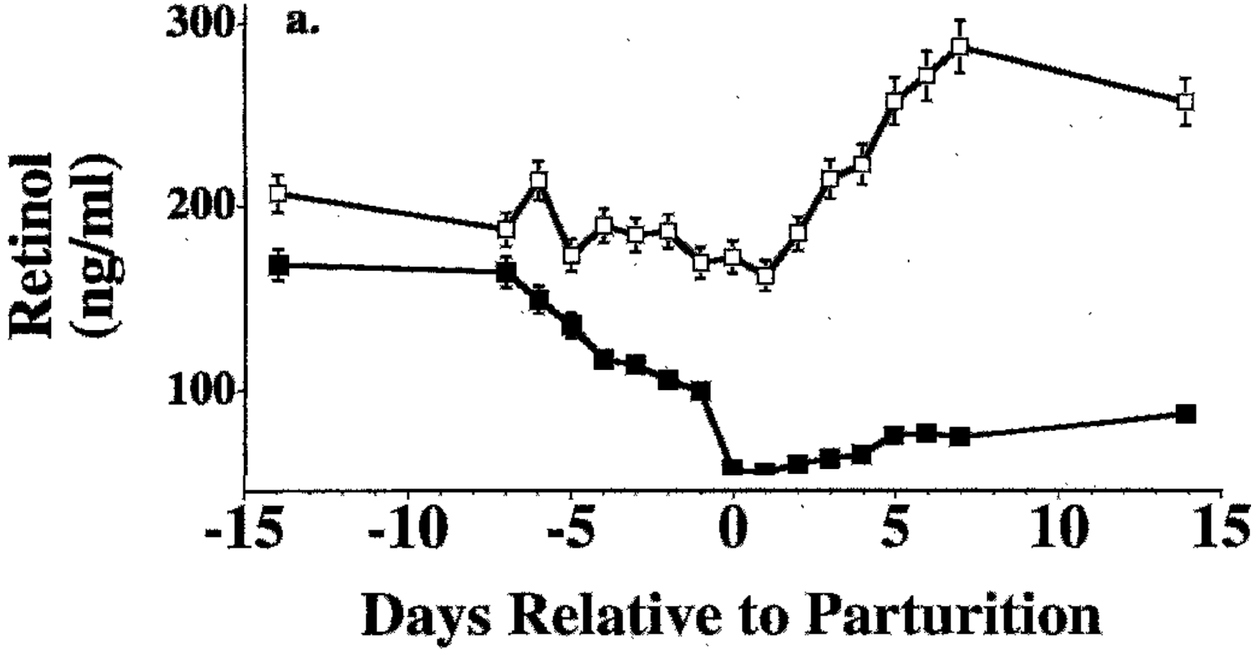
†EpiCentre, Institute of Veterinary, Animal, and Biomedical Sciences, Massey University, Palmerston North, New Zealand
‡Zinpro Corporation, 10400 Viking Dr., Ste. 240, Eden Prairie, MN 55344

OTM increased milk production by 0.93 kg [95% confidence interval (CI) = 0.61 to 1.25], milk fat by 0.04 kg (95% CI = 0.02 to 0.05), and milk protein by 0.03 kg (95% CI = 0.02 to 0.04) per day. No effect on SCC.

OTM reduced days open (weighted mean difference = 13.5 d) and number of services per conception (weighted mean difference = 0.27) in lactating dairy cows. The risk of pregnancy on d 150 of lactation was greater in cows fed OTM (risk ratio = 1.07), but OTM had no significant effect on the interval from calving to first service and 21-d pregnancy rate
Supplementation effect was increased when fed prior to calving and after calving

on

Mammary Influenced Vitamin Status



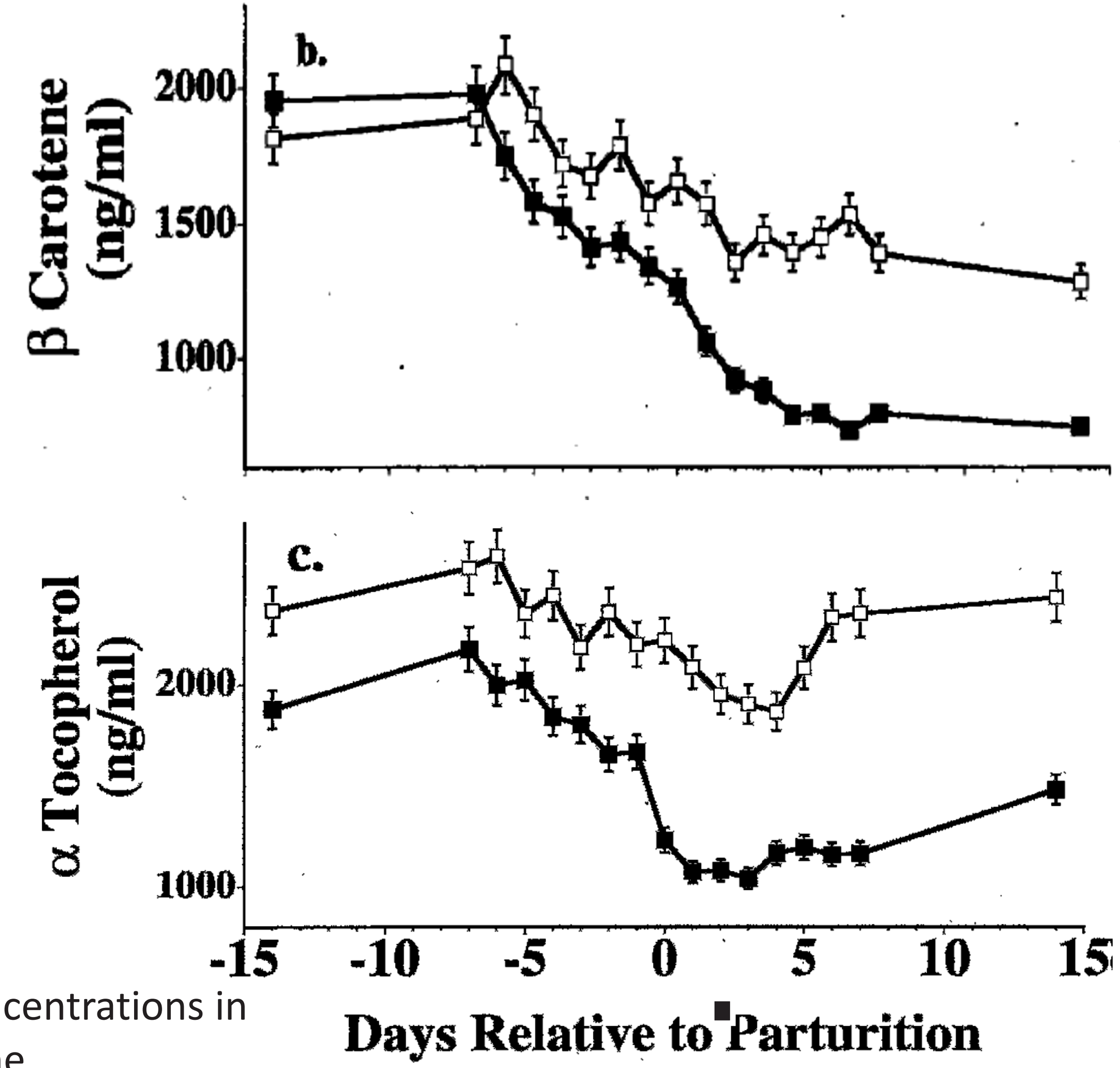


Figure 4. Plasma retinol (a), *β*-carotene, and *α*-tocopherol concentrations in intact (n = 8;) and mastectomized (n = 10; □) cows during the periparturient period.

Goff et al., JDS 2002

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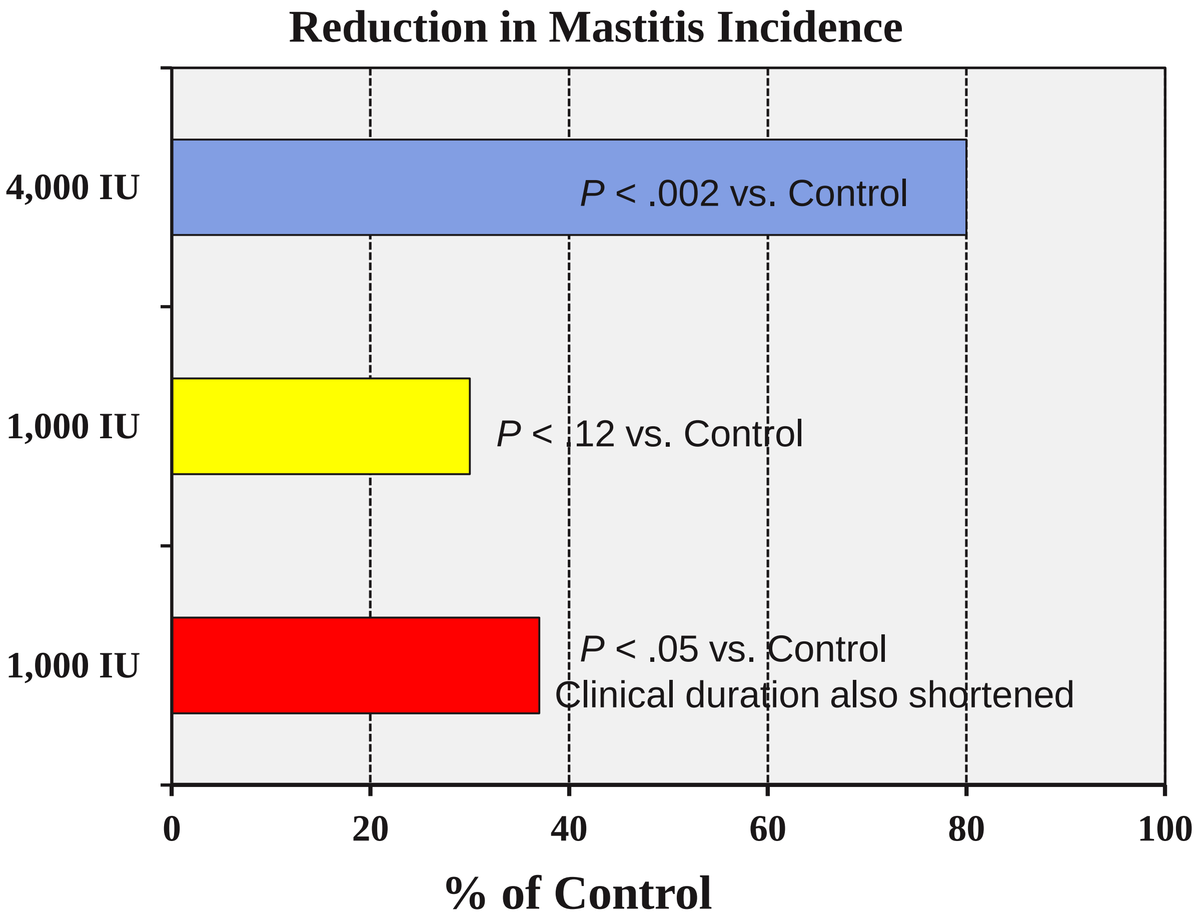
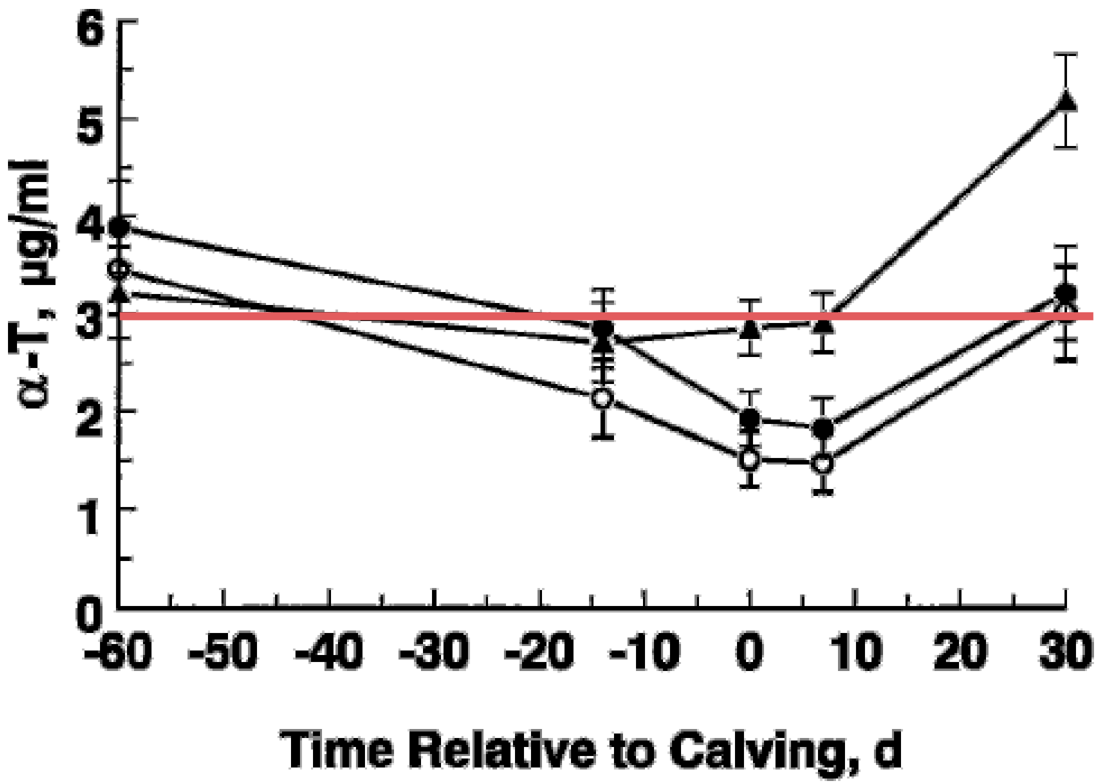
Vitamin E and Mastitis Prevalence

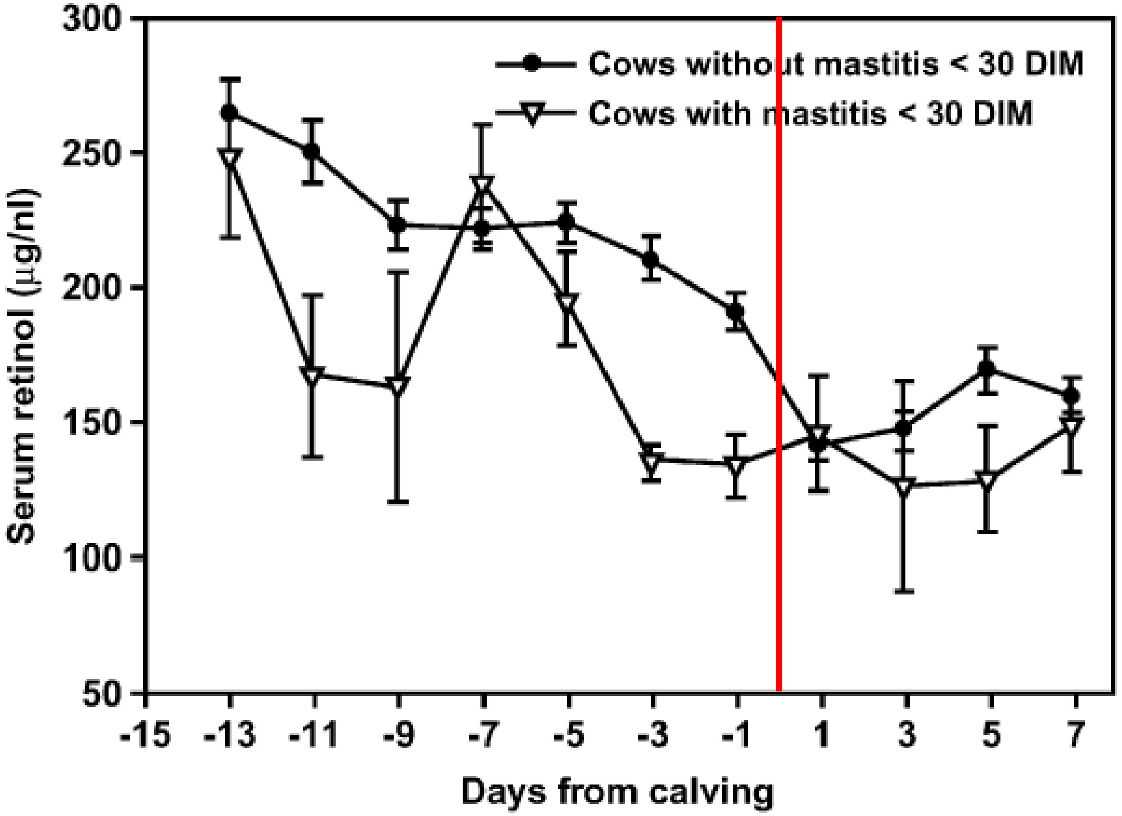


Cows with serum vitamin E concentrations < 3.0 µg/ml were 9.4x more (P<.02) likely to have mastitis



Weiss et al., J Dairy Sci 1997; Smith et al., JDS 1984

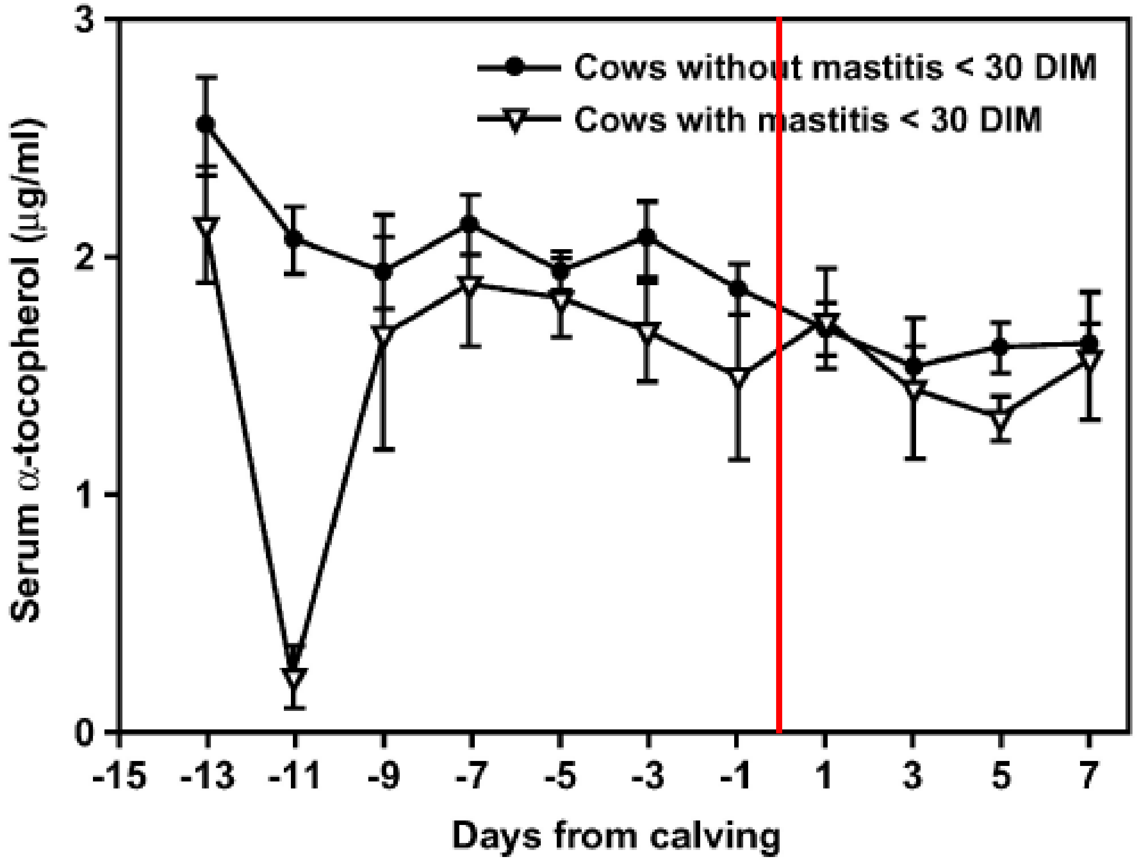
Vitamin A and Mastitis



- Survey study of 1057 cows over 1-year period
- Increasing serum retinol by 100 ng/mL was associated with 60% decrease in clinical mastitis in first 30 DIM
- Measured last week of pregnancy

LeBlanc et al., JDS 2004; 87:609-619

Vitamin E and Mastitis



- Serum vitamin E was lower in cows that had clinical mastitis within 30 DIM
- Increasing serum vitamin E 1 µg/mL reduced risk of retained placenta
- No effect on mastitis

LeBlanc et al., JDS 2004; 87:609-619

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Vitamin Status and Disease Risk

Table 1. Formulated ration summary from farms

Farm	Group	Vitamin A (kIU/kg of DM)	Vitamin E (IU/kg of DM)
1	Far-off dry	3.50	22.0
	Close-up dry	6.14	20.5
	Fresh cows	1.45	9.2
2	Far-off dry	2.80	20.1
	Close-up dry	5.80	32.4
	Fresh cows	0.91	9.9
3	Far-off dry	2.52	8.2
	Close-up dry	3.36	33.4
	Fresh cows	1.96	39.6
4	Far-off dry	7.92	82.8
	Close-up dry	12.58	94.1
	Fresh cows	7.52	46.3
5	Far-off dry	1.40	24.8
	Close-up dry	3.62	33.4
	Fresh cows	2.09	8.71

353 total cows in study

Strickland et al., J Dairy Sci 2021

Vitamin	Period	LSMean	SEM	Range
Retinol	Dry Off	312.74	16.15	102-528
(ng/mL)	Close-Up	283.68	16.20	76-598
	Lactation	250.50	16.26	53-764
β-Carotene	Dry Off	3.85	1.21	0.21-24.0
(μg/mL)	Close-Up	2.68	1.21	0.1-14.2
	Lactation	1.22	1.21	0.1-6.4
α-Tocopherol	Dry Off	4.69	1.09	0.8-13.1
(μg/mL)	Close-Up	3.00	1.09	0.4-11.1
	Lactation	1.44	1.09	0.0-7.1

- Lower retinol concentrations associated with hyperketonuria, uterine diseases (RFM, Metritis)
- No associations with lameness or mastitis
- Cause and effect???
- Lower retinol concentrations may be more a consequence of *increased inflammatory response*

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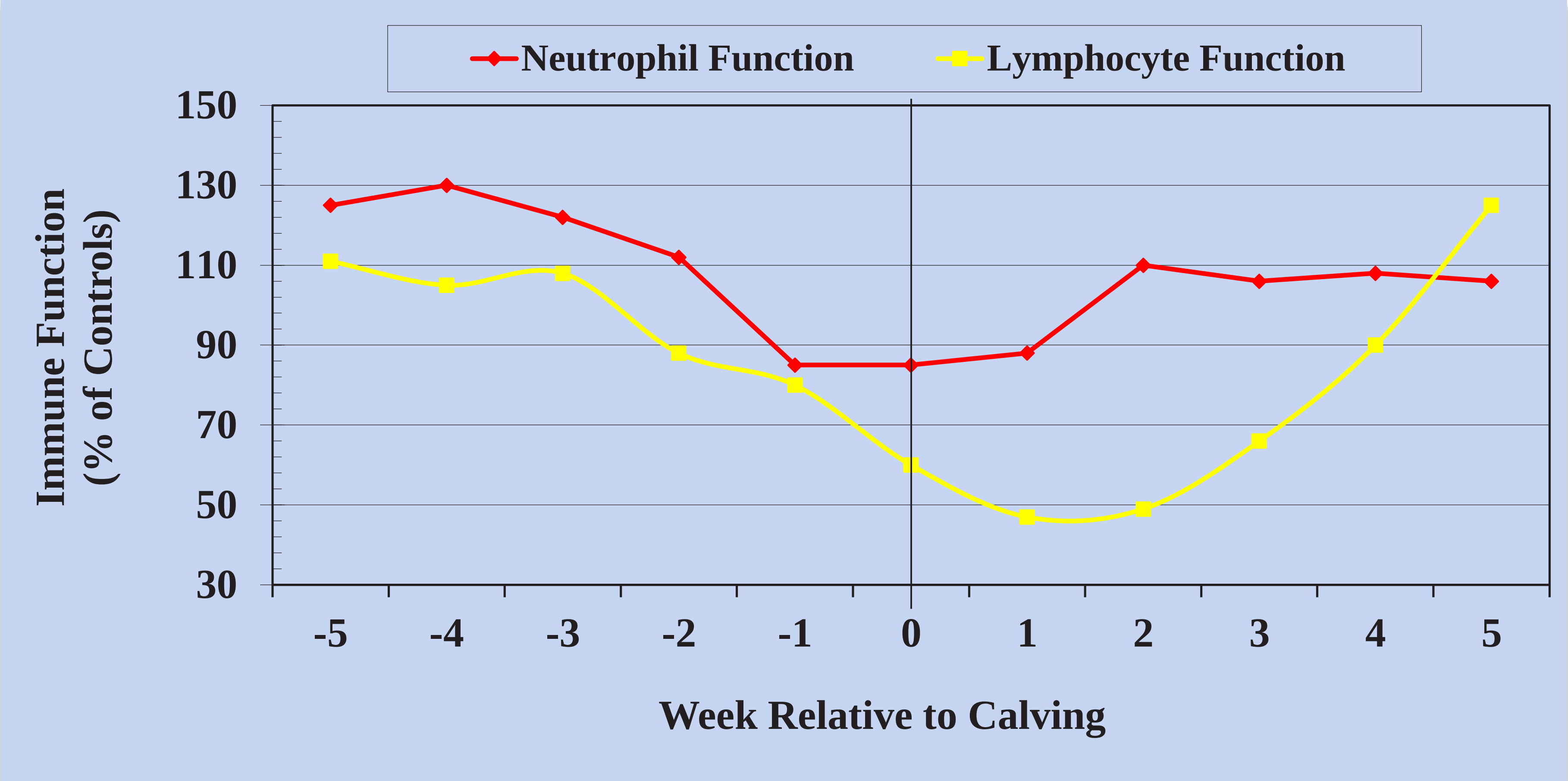
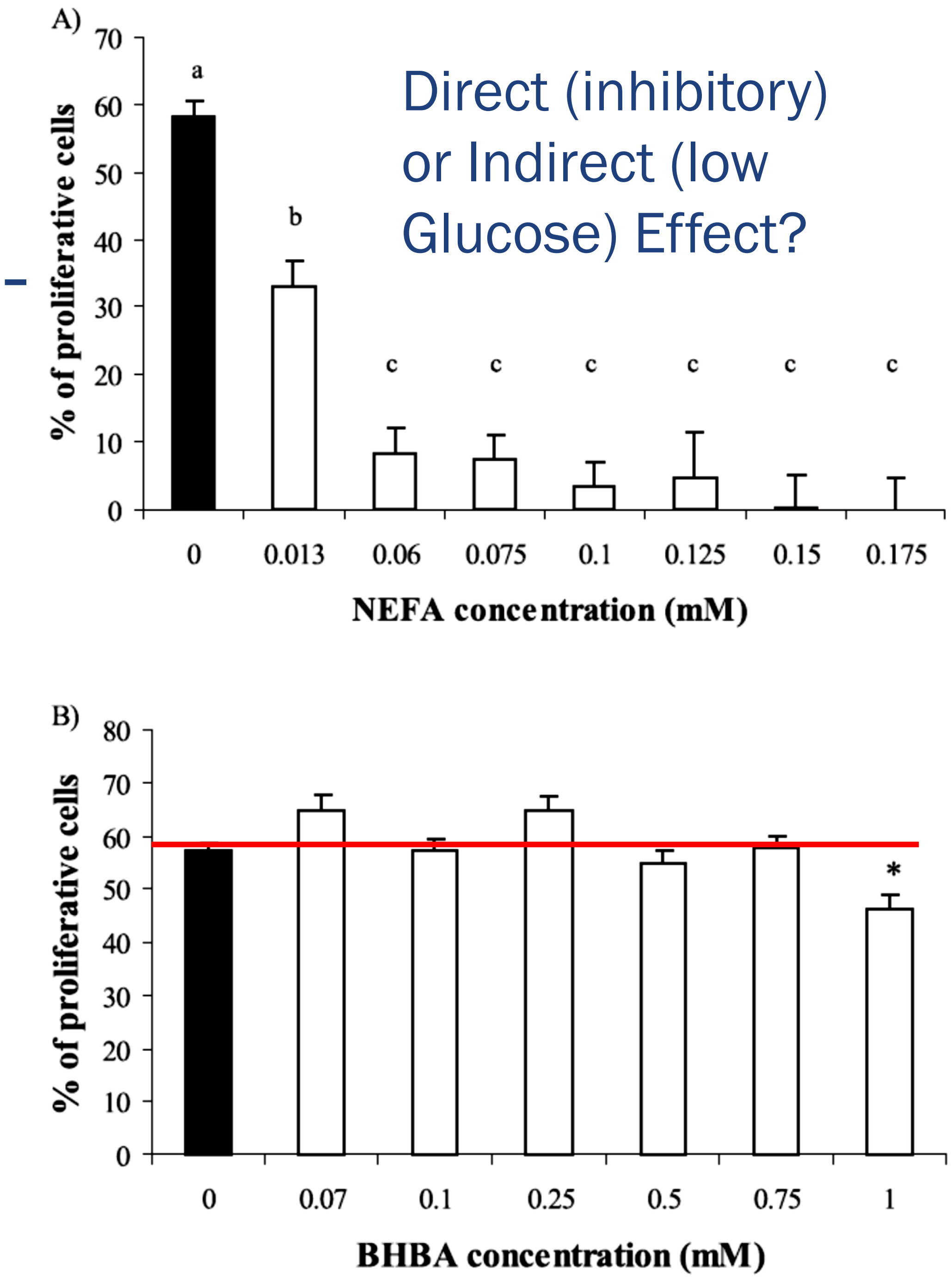
Immune Cell Functional Changes



Goff and Horst, JDS 1997;80:1260

What is the Cause of Impaired Immunity?

- Studies using mastectomized cows but allowed to go through parturition *(Kimura et al., 1999, 2002; Nonnecke et al., 2003)*
  - Some decline in neutrophil function in mastectomized cows, but greater response in those cows initiating lactation
  - Mastectomized cows had only moderate increases in NEFA
  - Changes in steroid profiles (estrogen, progesterone, glucocorticoids) can result in some alteration of immunity
  - Primary immunosuppressive factor is the increased metabolic demands of early lactation
- Inter-linking of metabolic diseases and immune dysfunction



Impact of Negative Energy Balance

- Graphs show the impact of either NEFA or BHB on peripheral blood mono-nuclear cells in their ability to proliferate when stimulated by concanavalin-A
- Increased NEFA and BHB as a result of negative energy balance have adverse effects on the immune response
- Some long-chain fatty acids are considered to have inhibitory effects on lymphocytes

Lacasse et al., Res Vet Sci 2018

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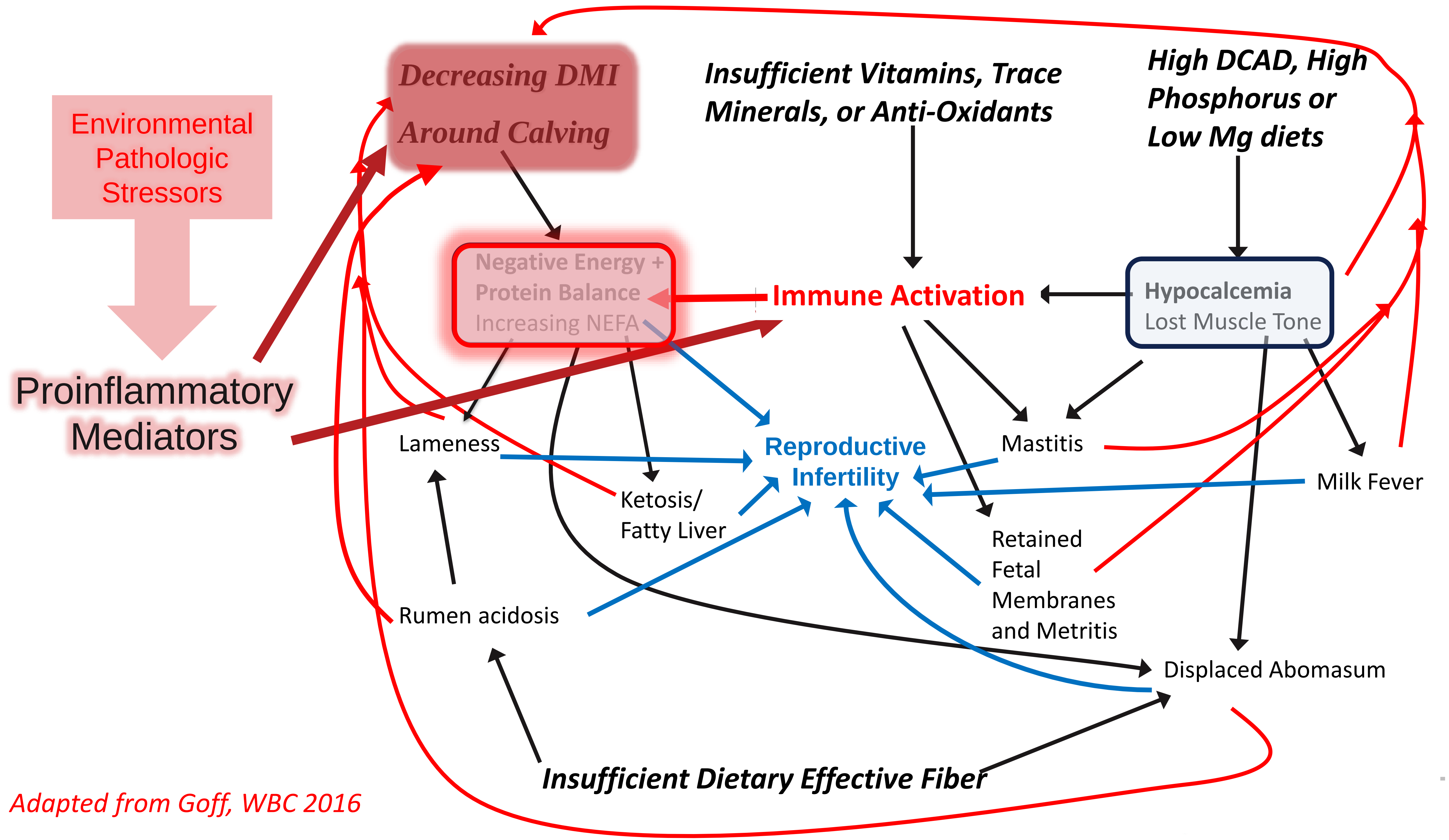
Transition Homeorhetic Nutrient Prioritization

<u>Postpartum</u>

- Maintenance
- Activated immune response
- Lactation
- Frame Growth
- Reserves
- Reproductive Cyclicity
- Pregnancy

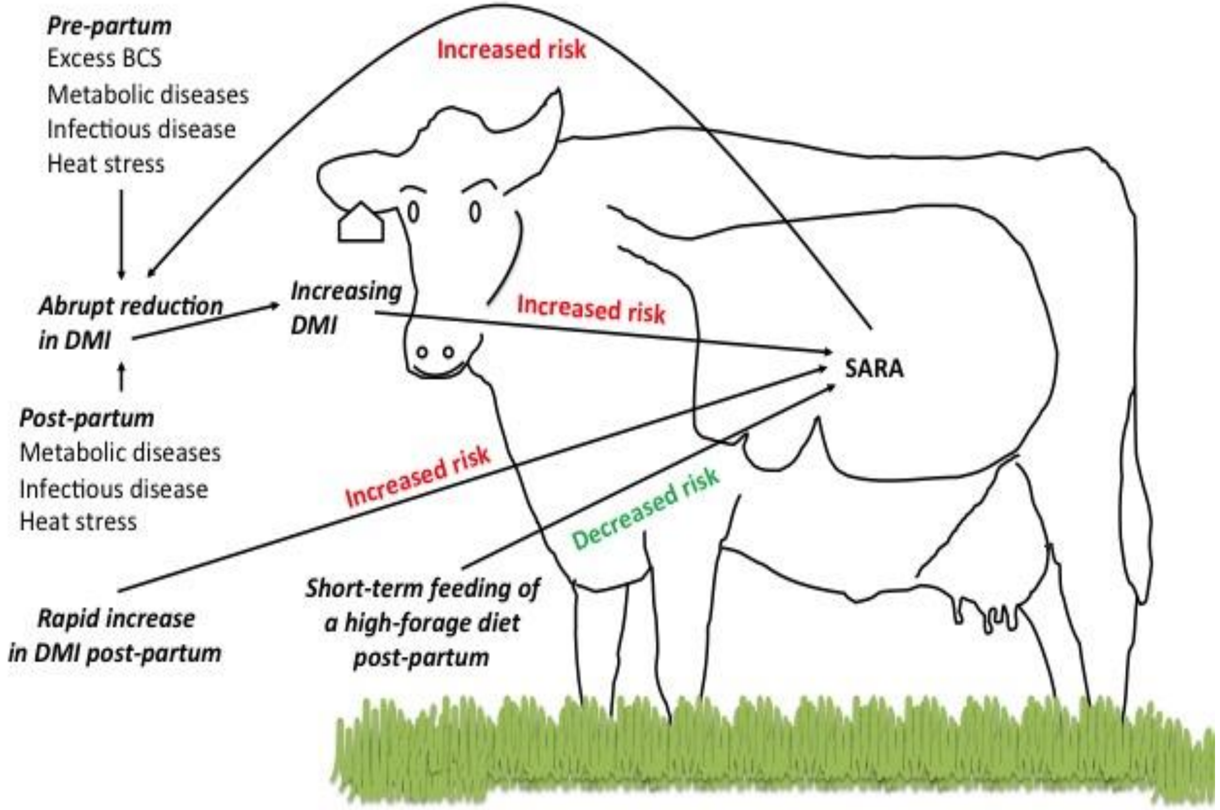
- Lactation places high nutrient demand at the expense of body reserves
- Mobilized body reserves constrain the resumption of reproductive activity
- Activated immune response has high priority and will increase maintenance leading to further adverse impacts on lactation, metabolism and reproduction

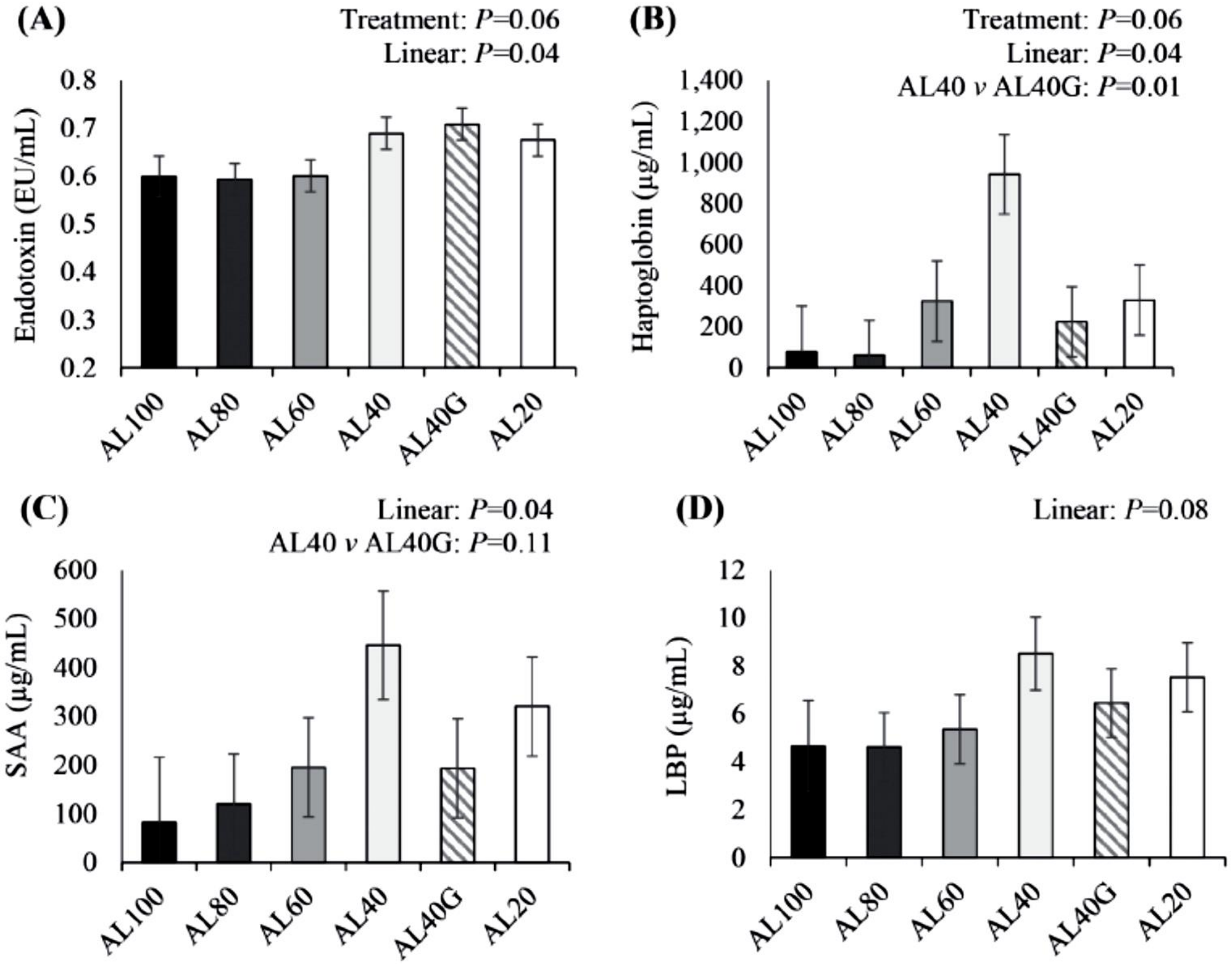
Extrapolated from Bauman and Currie, J Dairy Sci 1980



Adapted from Goff, WBC 2016

Low DMI Increases Inflammatory Risk

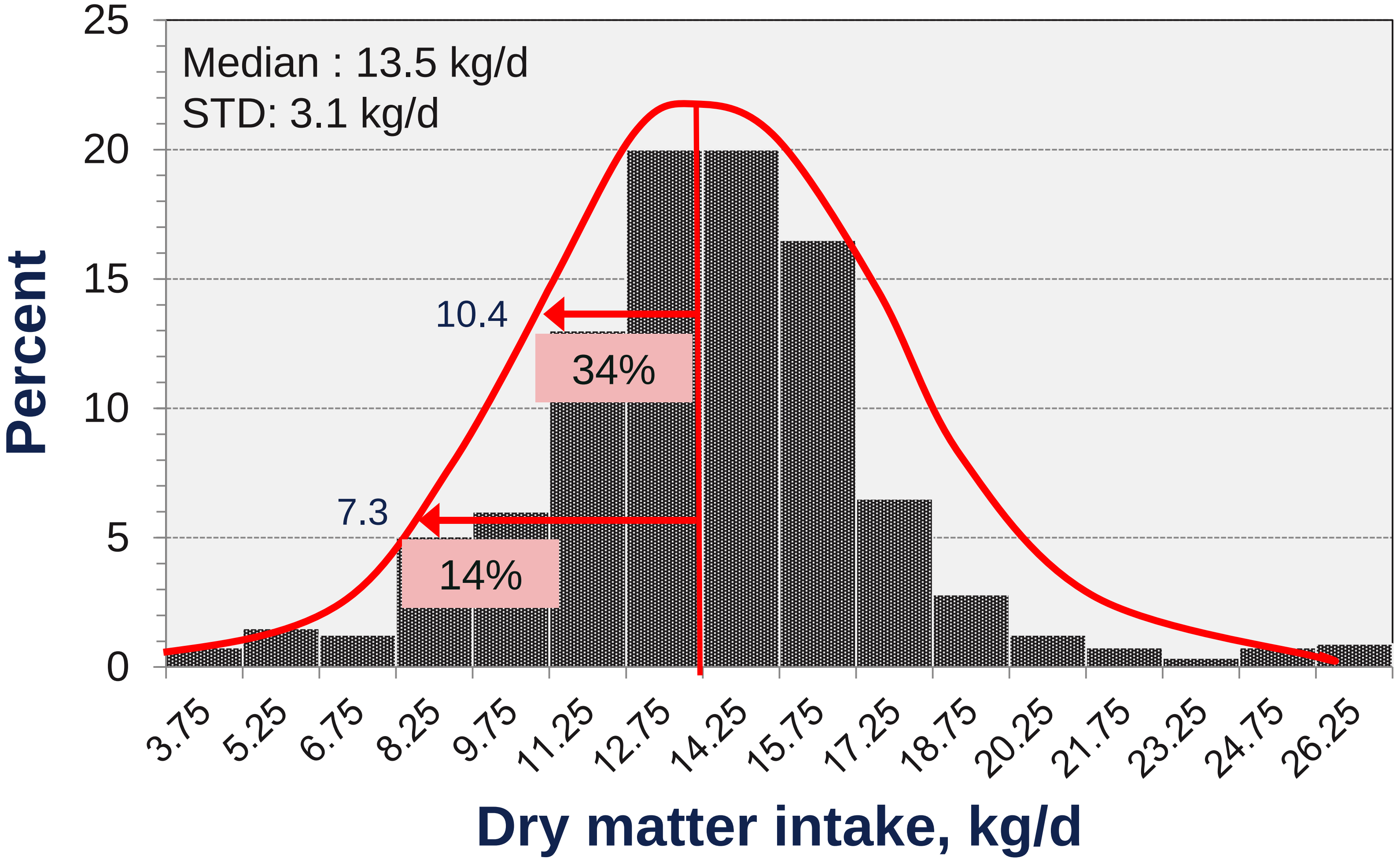




Penner, 2019

Kvidera et al., 2017; JDS

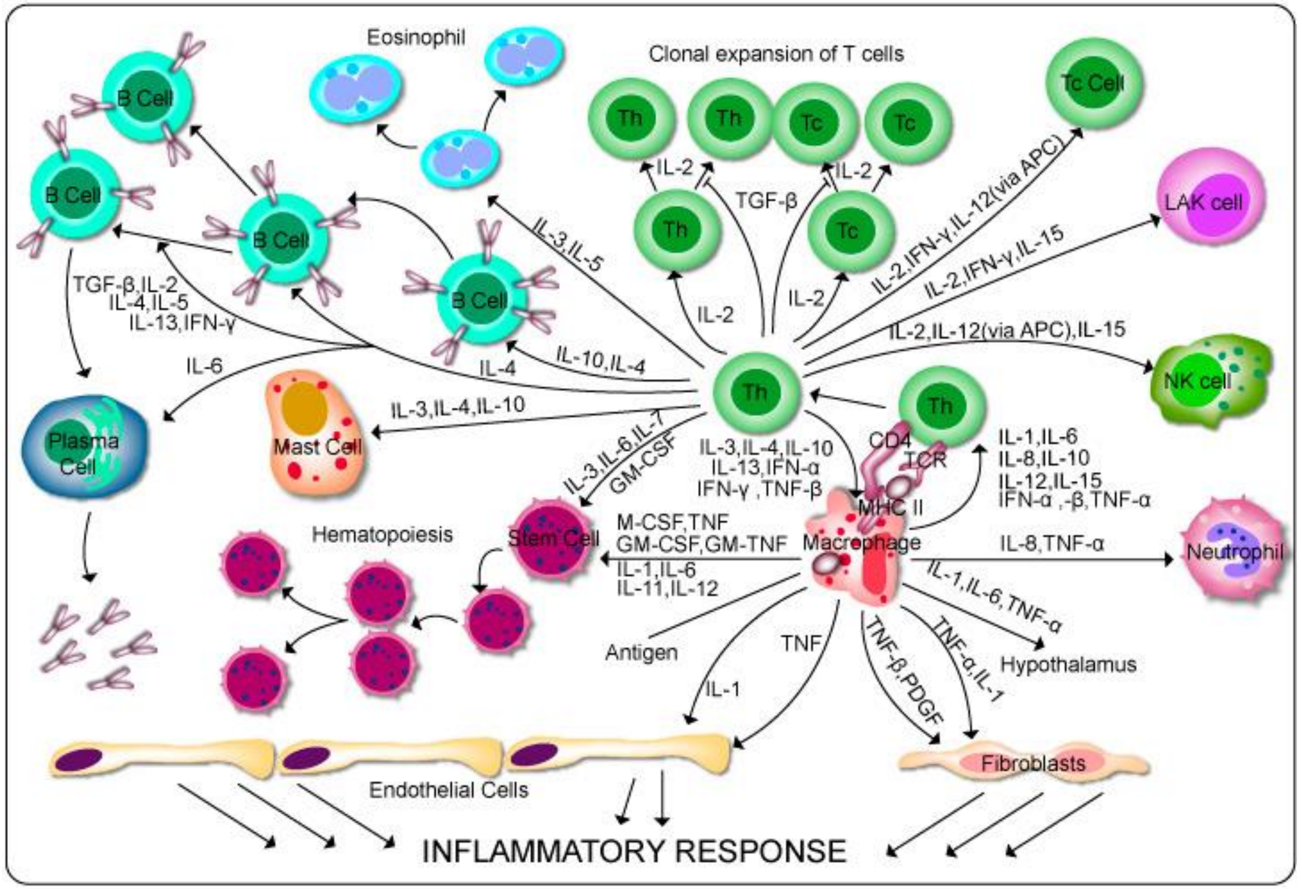
Daily Intake Variation Before Calving



Van Saun, 1993

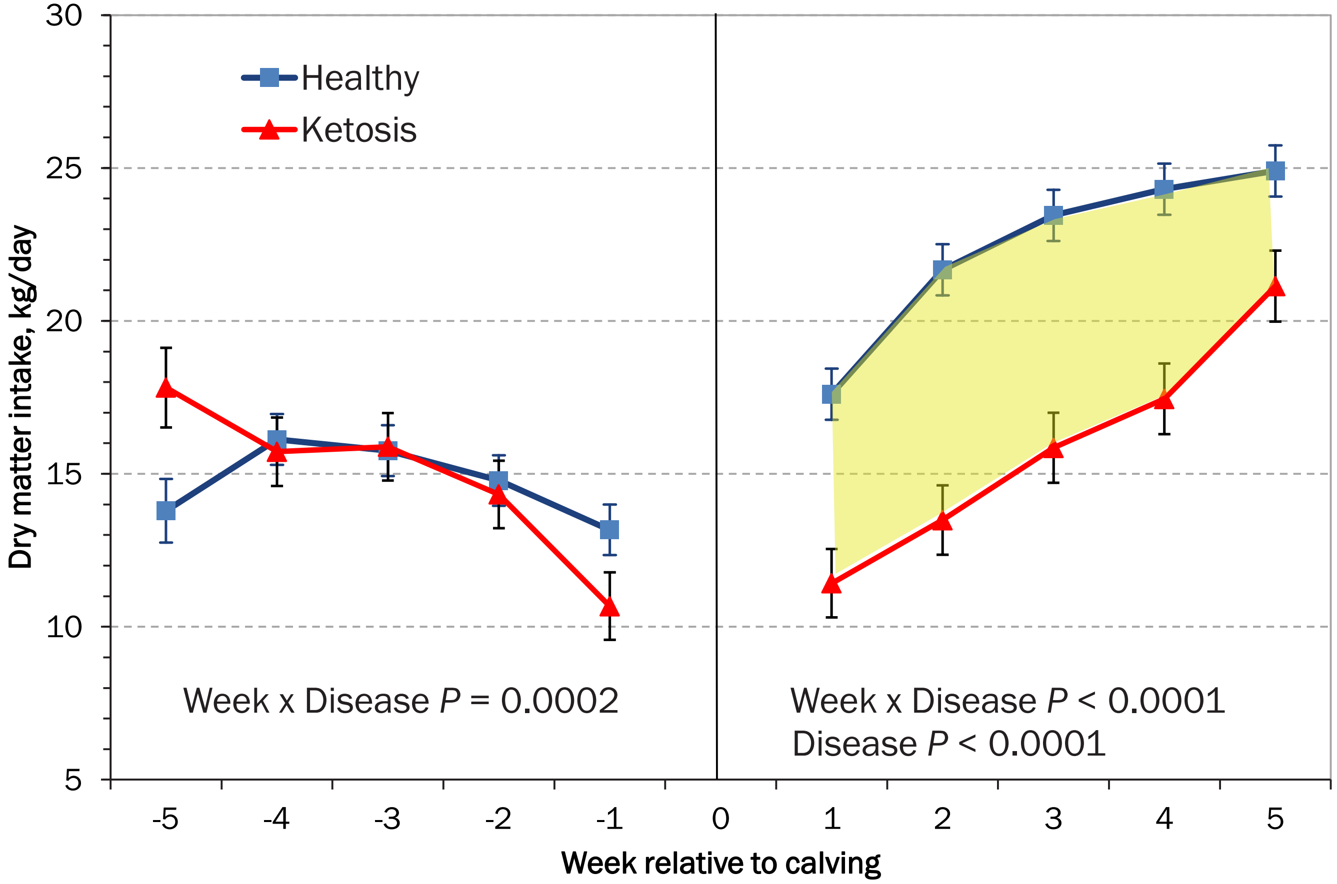
What is the cost of maintaining an immune response?

- The ability to mount an immune response or maintaining a competent immune system is nutritionally demanding
- Requires trade-off decisions among competing nutrient demands for other physiologic functions
- Large consumer of glucose and amino acids



Lochmiller RL and Deerenberg C OIKOS 2000

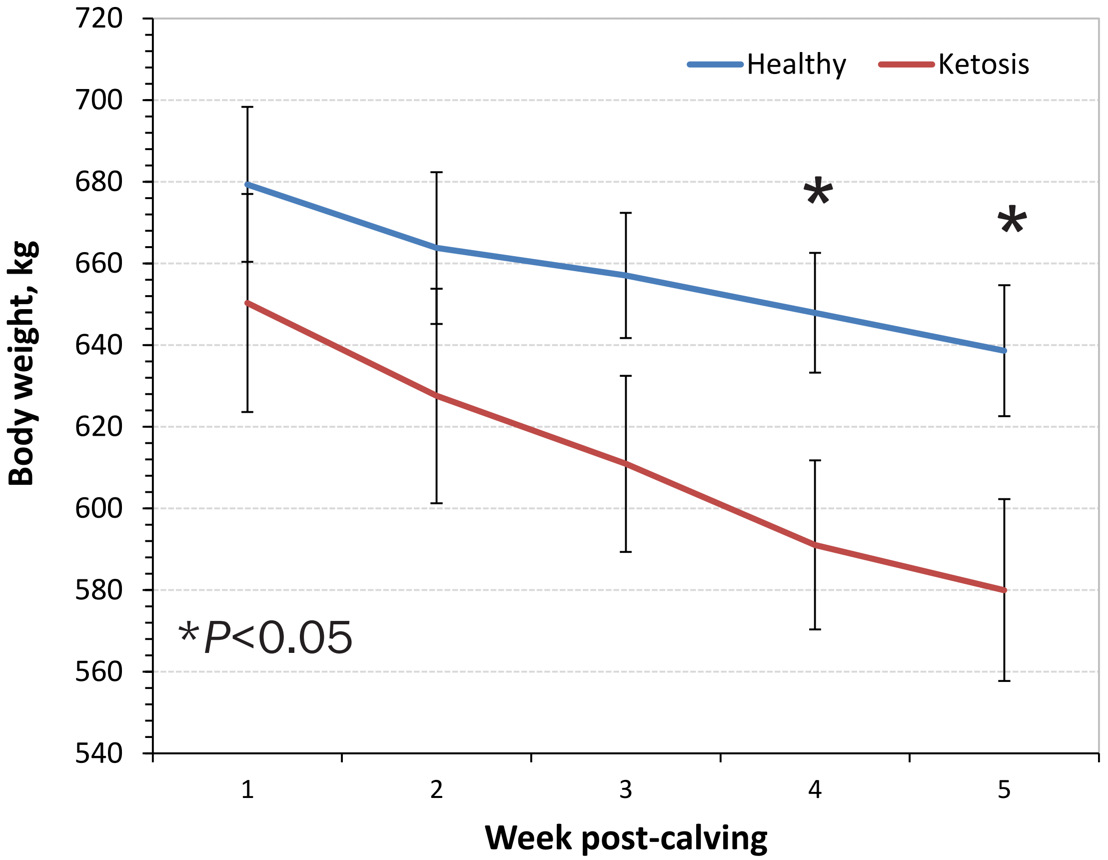
Dry Matter Intake and Ketosis

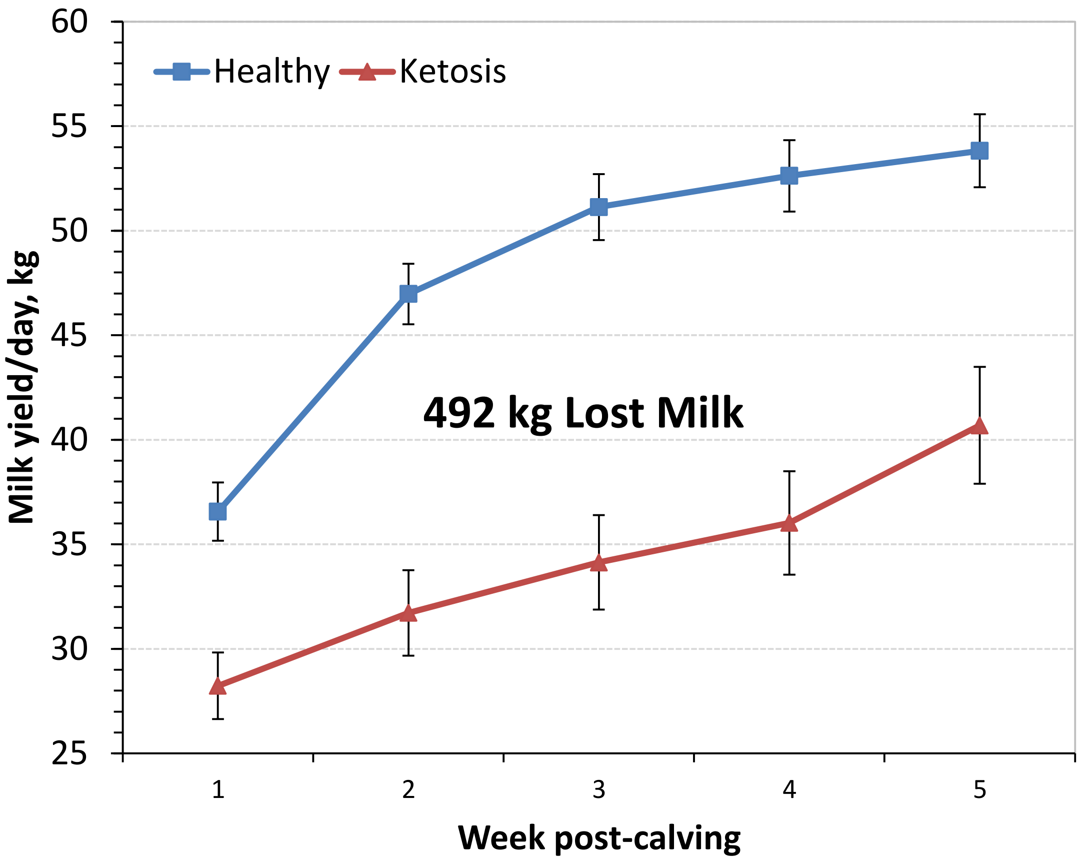


- Total DMI loss in first 5 weeks was 228 kg
- Using dietary energy content, total energy loss would account for:
  - 0.88 Body Condition Score loss
  - 470 kg 4% milk yield loss
- What is causing the prepartum intake drop?

Van Saun, Vet Clinics NA: Food Animal Practice 2023

BW and Milk Yield Responses





Van Saun, Vet Clinics NA: Food Animal Practice 2023

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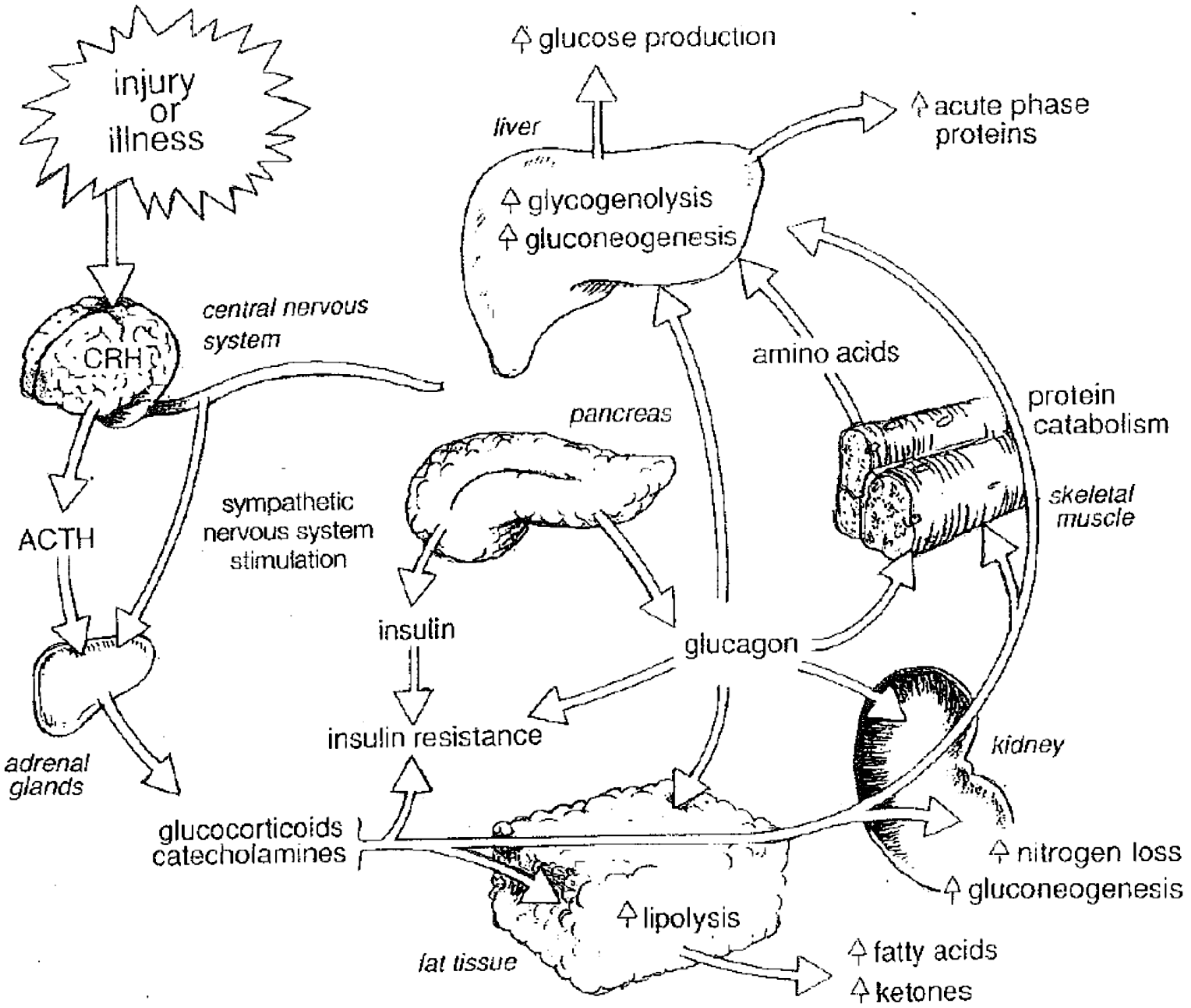

Hypermetabolism from Disease or Injury

Hormonal regulators in response to an injury or illness and corresponding metabolic responses in various tissues

Primary effects are to stimulate release of fatty acids for energy and increase protein catabolism to release amino acids to support gluconeogenesis and acute phase protein response

All responses are occurring in a state where intake is reduced

Chandler et al., Cont Ed Pract Vet 1992

Physiologic Response to Inflammation

Phase	Duration	Role	Physiologic	Hormones
Ebb	< 24 hours	Maintenance of blood volume, Catecholamines	↓BMR, Temperature, O_2 consumption; ↑Heart rate, cardiac output; Vasoconstriction; Acute Phase Proteins	Catecholamines, Cortisol, Aldosterone ↑Proinflammatory cytokines
Flow				
Catabolic	3-10 days	Maintenance of energy	↑BMR, Temperature, O_2 consumption; Negative Nitrogen Balance (severity depends on ↑↑ proinflammatory cytokines)	↑Insulin, Glucagon, Catecholamines, and Cortisol with Insulin resistance
Anabolic	10-60 days	Replacement of lost tissue	Positive Nitrogen Balance	Growth hormone, IGF-1

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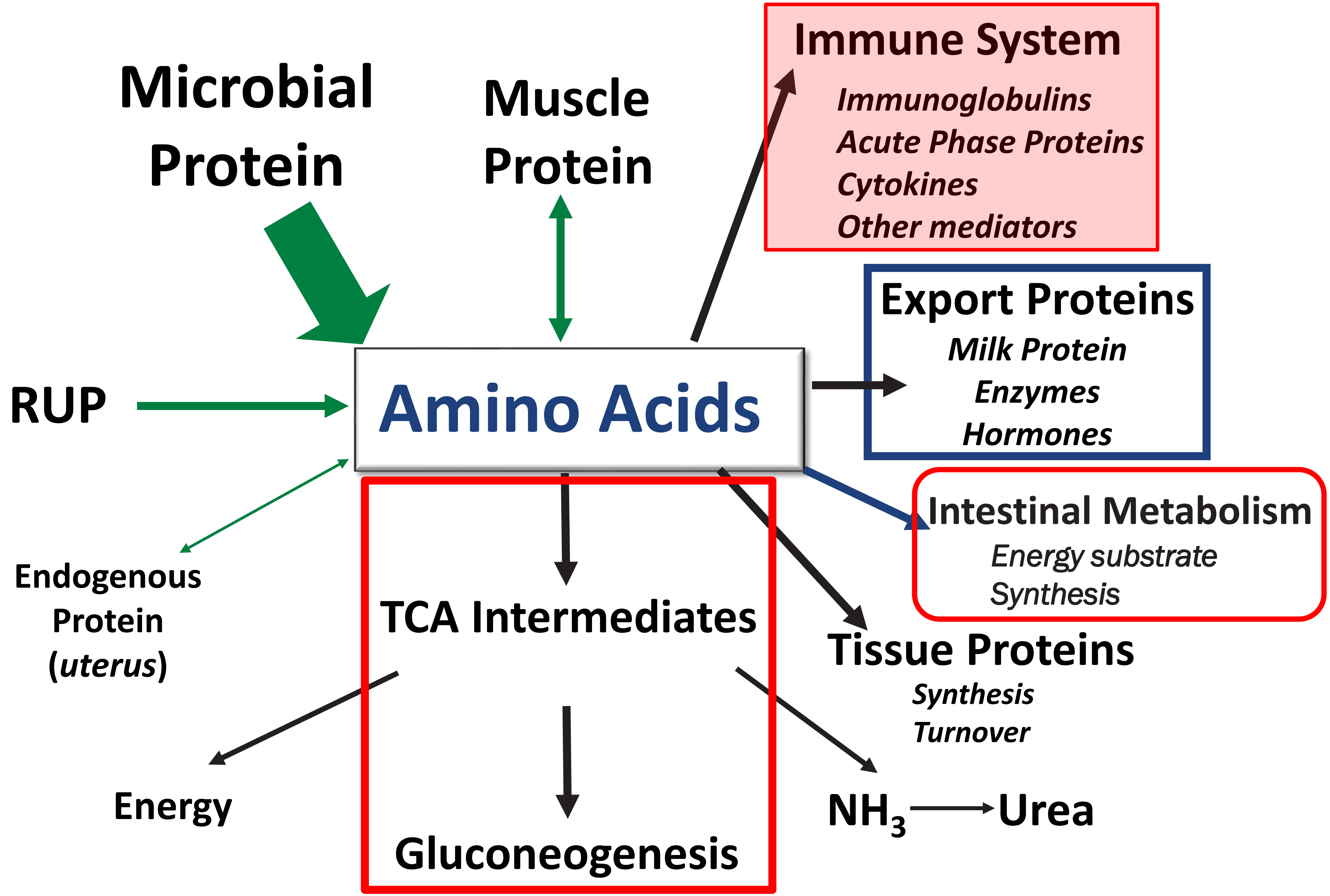
Energy and Protein Metabolism in Inflammation

Species	Immune Challenge	Resting Metabolic Rate
Human	Sepsis	+30%
	Sepsis	+30%
	Sepsis and injury	+57%
	Typhoid Vacc.	+16%
	Sickle Cell disease	+15%
Lab rat	IL-1 infusion	+18%
	Inflammation	+28%
Lab mouse	KLH challenge	+30%
Sheep	Endotoxin	
	Endotoxin	10-49%

Species	Immune Challenge	Protein Parameter	% Change
Pig	PRRS vacc.	BW gain	-21%
		Feed intake	-15%
Chicken	HVT vacc.	Feed intake	-3%
	SRBC	BW gain	-13%
	Endotoxin	BW gain	-18%
Human	Sickle cell	Prot. Catabolism	+32%
		Prot. Synthesis	+38%
	Sepsis	N excretion	+160%
	Sepsis/injury	Total Body Protein	-12%
Lab rat	Sepsis	Prot. Catabolism	+40%

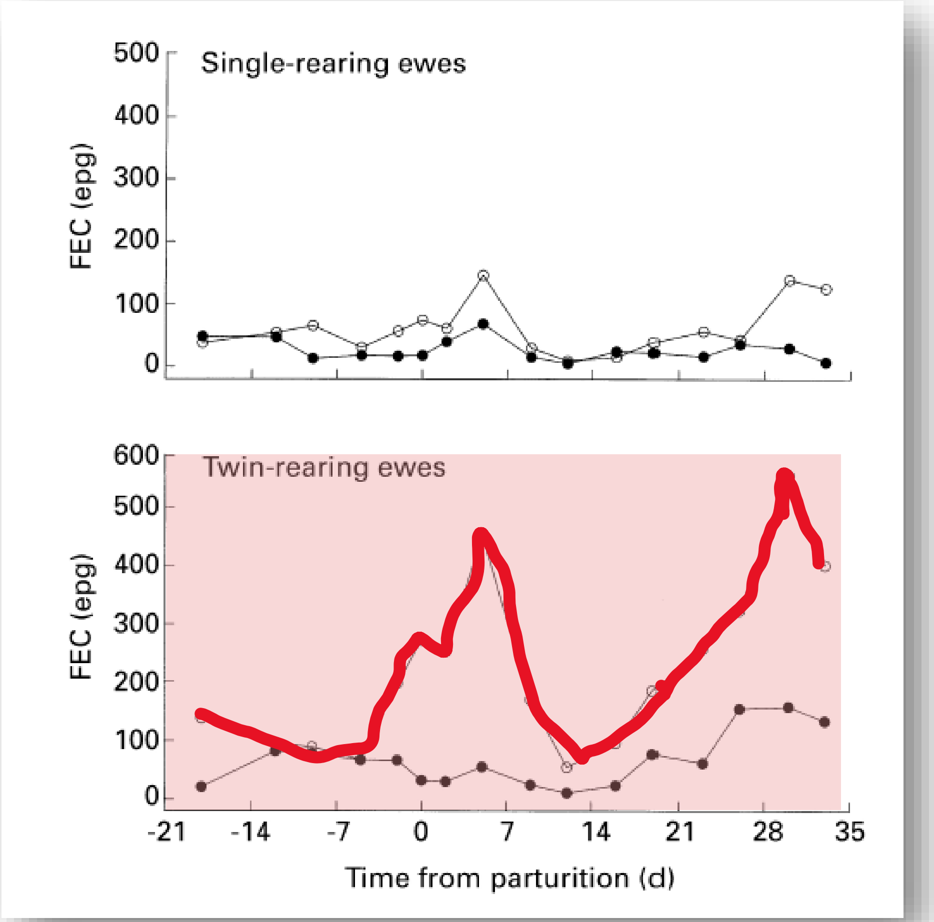
Lochmiller RL and Deerenberg C OIKOS 2000


Microbial Protein
Muscle Protein
Immune System
Immunoglobulins
Acute Phase Proteins
Cytokines
Other mediators
Export Proteins
Milk Protein
Enzymes
Hormones
RUP
Amino Acids
Intestinal Metabolism
Energy substrate
Synthesis
Endogenous Protein (uterus)
TCA Intermediates
Tissue Proteins
Synthesis
Turnover
Energy
Gluconeogenesis
NH_3
Urea
on

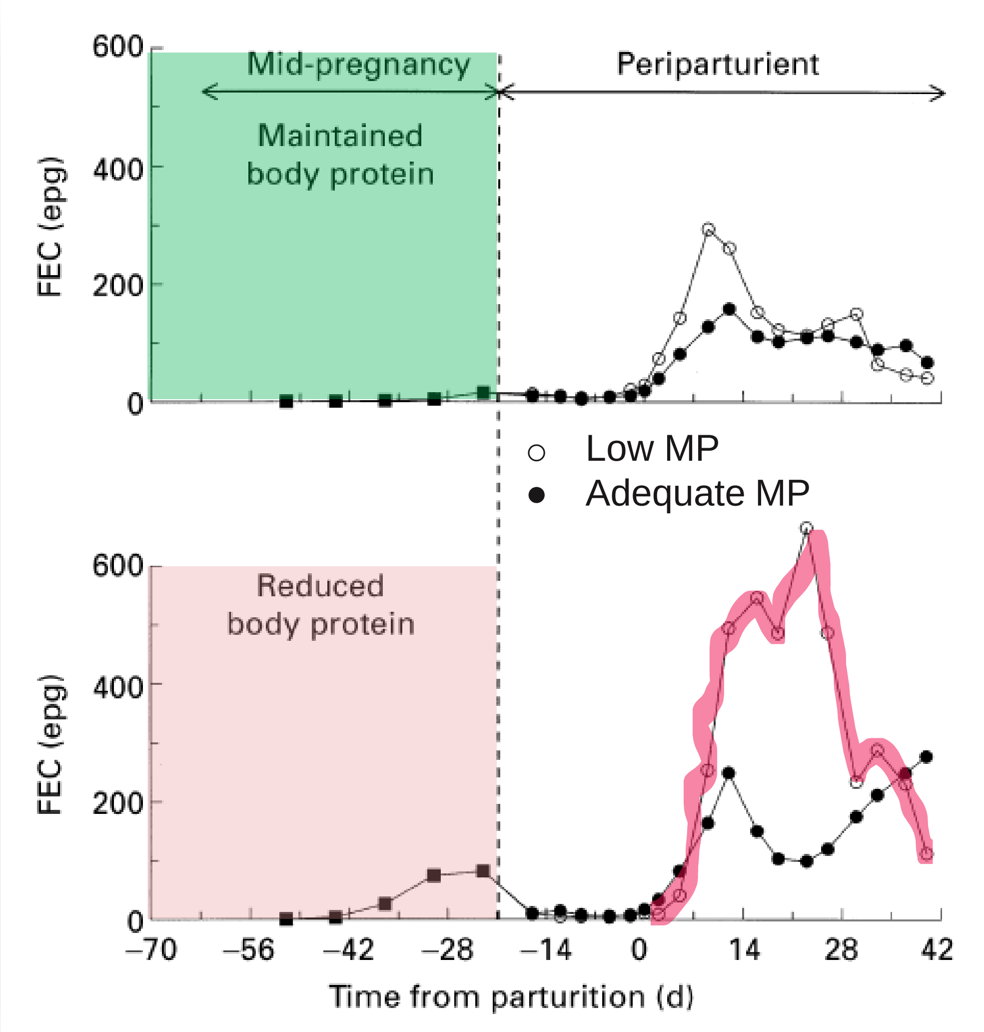
Nutrition and Parasite Control

- Improving protein and not energy status in late pregnancy improved GI immunity to parasites (*Jones et al., Intl J Parasit 2011*)
- Improved body protein status and increased dietary protein supply reduced fecal egg counts and improved immune status (*Houdijik et al., Vet Parasit 2000; Houdijk et al., Parasitology 2001*)
  - Diets provided either 85% or 130% of MP requirements

Effect of Protein on FEC



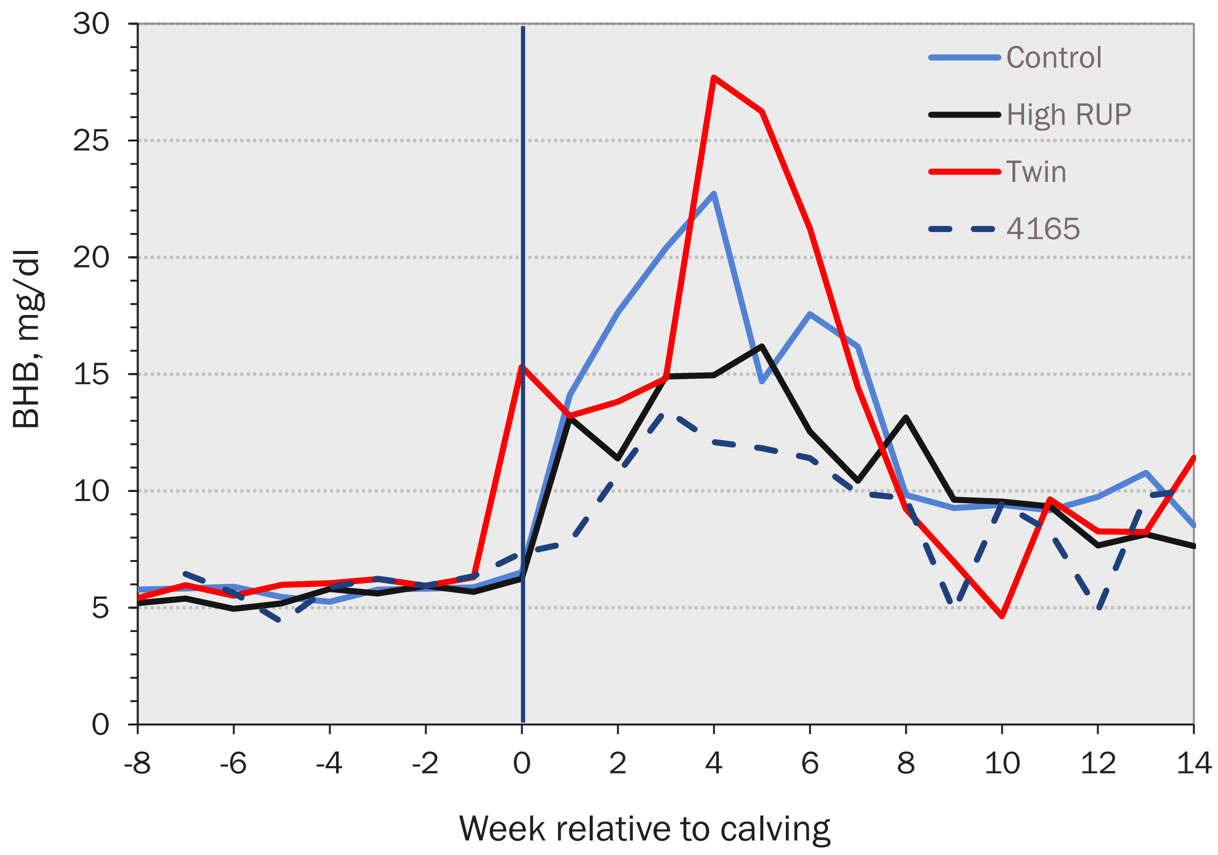
Fecal egg counts (FEC) in ewes either fed inadequate (○) or adequate (●) metabolizable protein during pregnancy.



Fecal egg counts (FEC) in ewes fed inadequate (○) or adequate (●) metabolizable protein during periparturient period following mid-pregnancy feeding to either maintain or reduce body protein.

Houdijk et al. Proc Nutr Soc 2001

Effect of Protein on Ketogenesis



- Prepartum diets differed in amount of protein provided by RUP sources
- Control group cows experienced 31% clinical ketosis
- High RUP group did not present with clinical ketosis
- Cows with twins had clinical ketosis and other concurrent diseases
- Cow 4165 had twins, but fed the High RUP diet prepartum
- Postpartum diets were isocaloric and isonitrogenous

Van Saun, PhD Thesis 1993

Role of Protein in Disease?

Measured Concentration in Fresh Cows 3-21 DIM

Albumin	Healthy	Abnormal
≤ 30 g/L	33.3% (6/18)	66.7% (12/18)
> 30 and ≤ 35 g/L	38.9% (21/54)	61.1% (33/54)
> 35 g/L	67.7% (21/31)	32.3% (10/31)

Overall Model: P<0.02

Methionine Supplementation

Close-up Methionine Supplementation

- Reduced inflammatory and increased antioxidant capacity *(Osorio et al., 2014; Batistel et al., JDS 2017; Sun et al., 2016; Zhou et al., 2016)*
- Improved milk yield, components, energy balance *(Osorio et al., 2013)*
- Upregulation of metabolic regulators of lipid metabolism and immune function *(Osorio et al., 2016)*

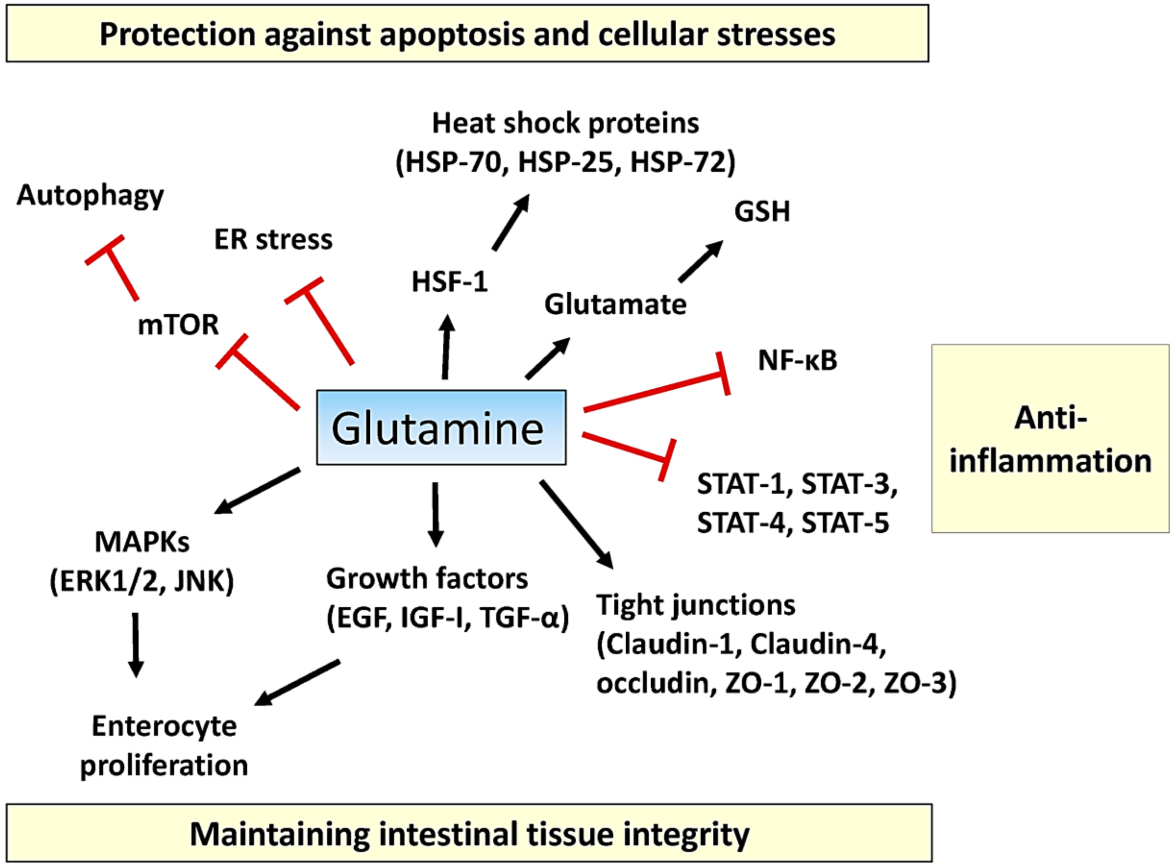
Additional MHA (+20 g/d) on balanced diet

- Reduced body protein mobilization
- Improved milk and feed intake *(Phillips et al., 2003)*

High CP ± RP-Meth

- Increased intake, lower plasma IL-1 concentration, reduced inflammatory status compared to low CP diet (prepartum – early lactation)
- Methionine addition increased prepartum insulin *(Cardoso et al., JDS 2021)*

Intestinal Amino Acid Metabolism



- Glutamine is major substrate utilized by intestinal cells (30% of total body)
- Intestine utilizes most of body glutamate and aspartate
- 30-50% of dietary arginine, proline and branched-chain amino acids consumed by the intestine
- Amino acids serve as energy and protein synthesis substrates
- Role in intestinal integrity and immune function

Kim and Kim, Internat J Molecular Science 2017

Amino Acid Composition (g/100 g protein)

Amino Acid	Cattle[1]	Sheep[2]	Amino Acid	Cattle	Sheep
Ala	6.47	3.4-6.2	Lys	8.10	8.1-11.5
Arg	4.72	4.6-5.3	Met	2.29	1.6-2.5
Asp	11.99	11.2-12.9	Phe	5.43	4.9-5.7
Cys	1.67	2.0-2.6	Pro	3.74	3.4-4.0
Glu	13.02	12.7-14.1	Ser	4.43	4.1-4.7
Gly	5.22	4.9-6.5	Thr	5.34	5.2-6.6
His	1.88	1.6-2.1	Trp	1.18	NA
Ile	5.71	5.4-6.2	Tyr	5.18	4.4-5.1
Leu	7.93	7.4-8.3	Val	5.71	5.3-6.5

- Amino acid composition of mixed rumen bacteria and protozoa
- Glutamine and Glutamate are found in the highest proportion
- Can increasing microbial protein improve intestinal integrity and immunity?

[1]*Sok et al., J Dairy Sci 2017*
[2]*Cao et al., Amino Acids in Nutrition and Production of Sheep and Goats (Wu, G, ed., Amino Acids in Nutrition and Health), 2021*

Failing to Prepare is Preparing to Fail!

- Ensure appropriate nutrient balance of the dry cow diet
  - Energy and Proteins status
  - Maintain postpartum calcium homeostasis
  - Intake capacity relative to group intakes
- Ensure adequate or increased intake of critical immune supportive nutrients
  - Trace minerals – organic vs. inorganic sources
  - Vitamins – especially A, D, and E
  - Other antioxidant compounds? Botanicals?
- Address environmental management issues to optimize nutrient intake and reduce marked declines in DMI before calving

Postpartum Immune Response – Attenuated or Activated?

- Primary focus to minimize stressors accentuating inflammatory response
    - Heat stress abatement
    - Socialization factors – pen changes
    - Dietary issues of SARA, mycotoxins leading to depressed intake
- Minimize prepartum body protein mobilization coupled with additional postpartum dietary MP or methionine
- Formulate diets to encourage increasing intake postcalving providing energy and microbial protein to support production and immune response
- Additional antioxidants in fresh diet? Namely vitamins A and E?

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Thank You for Your Attention!

Questions?

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