

# LIFESTART Science

Our journey to unlock the potential of dairy calves

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# In 2012 Trouw embarked in a fascinating research journey



**LIFESTART**  
SETS LIFE PERFORMANCE



- More than 30 peer review publications
- 3 patent families
- 4 new products
- New feed protocols and formulations



# Glossary of terms

**EPIGENETICS**

**PHENOTYPICAL  
PLASTICITY**

**METABOLIC  
PROGRAMMING**

**SILVER SPOON  
EFFECT**

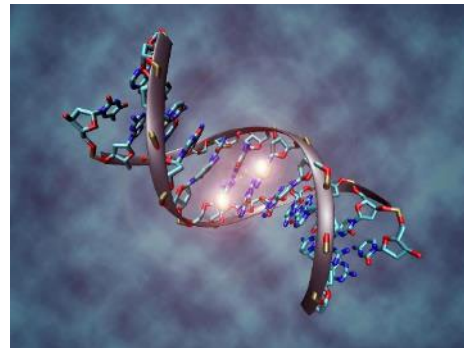
**METABOTYPE**

**THRIFTY  
PHENOTYPE**

**WINDOW OF  
OPPORTUNITY**

# Hunger epigenetics

- “Hongerwinter” Dutch famine Amsterdam 1944/45
- Long term correlation with metabolic syndrome
- Great Chinese famine 1958 and Biafra 1968



Picture Christoph Bock, Max Planck Institute

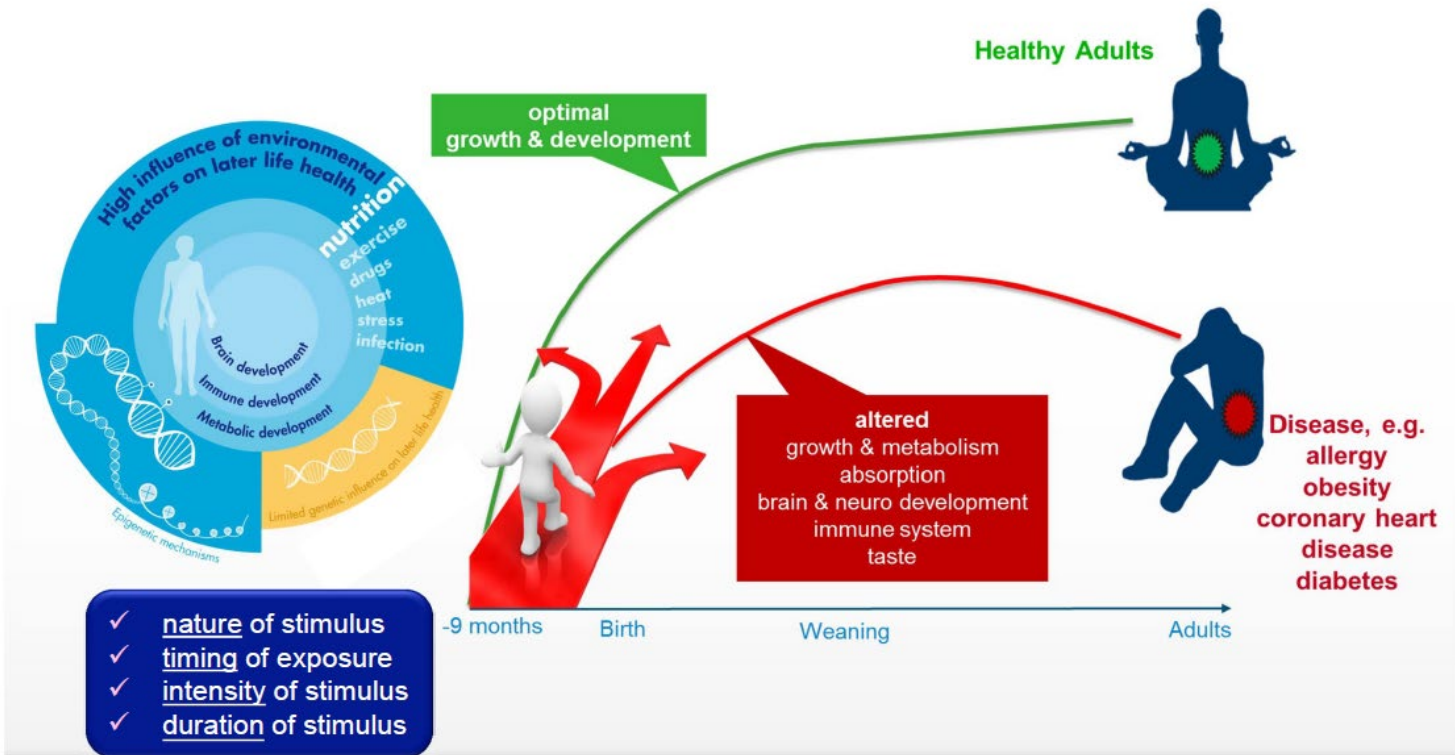


Picture Menno Huizinga

- Effects mediated by difference in DNA methylation associated to perinatal environment

# IMPACT ON LATER LIFE HEALTH

CRITICAL WINDOW OF OPPORTUNITY

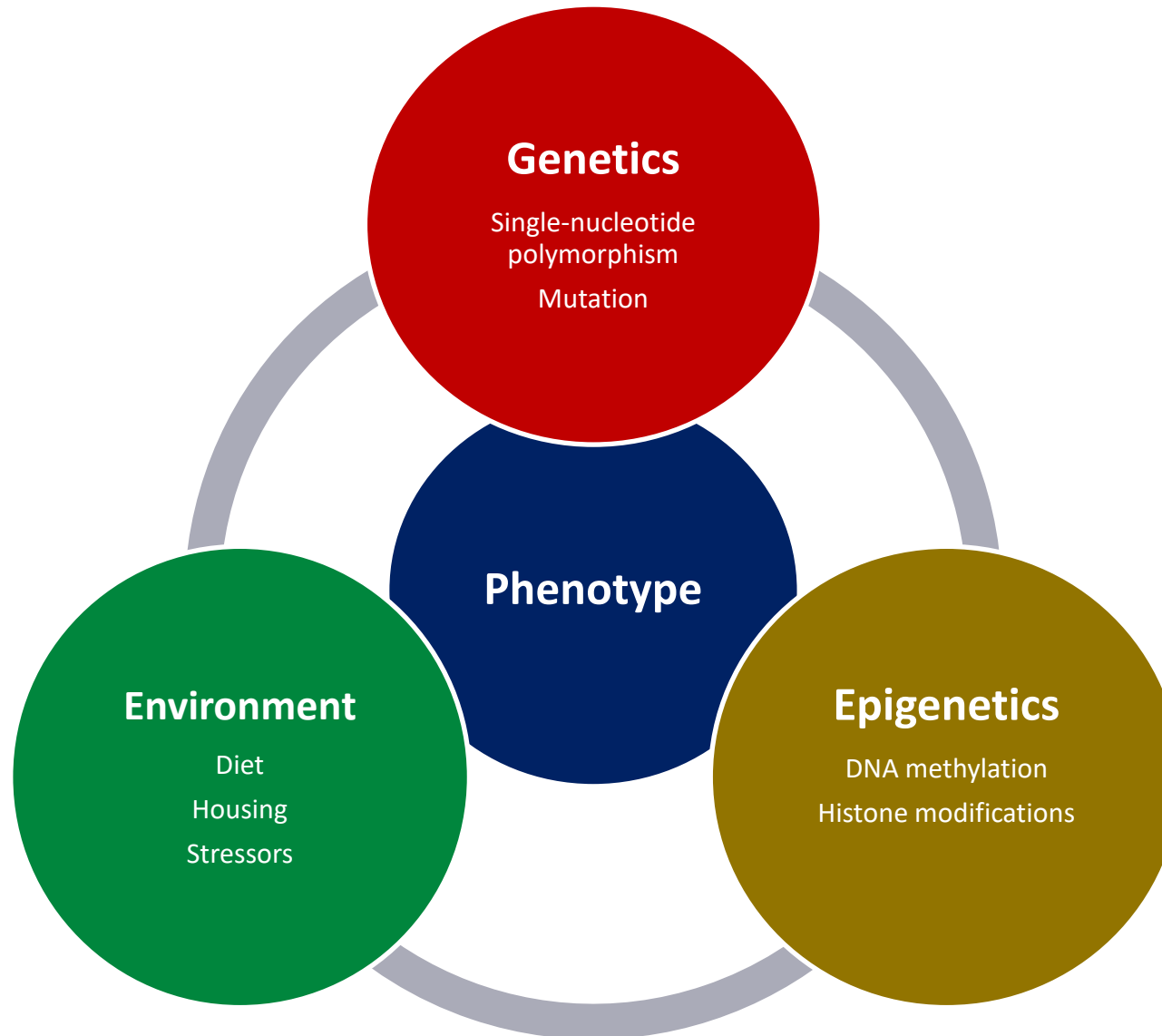


Nutricia Research ©

NUTRICIA  
RESEARCH



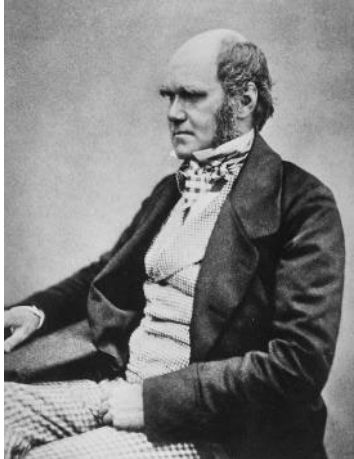
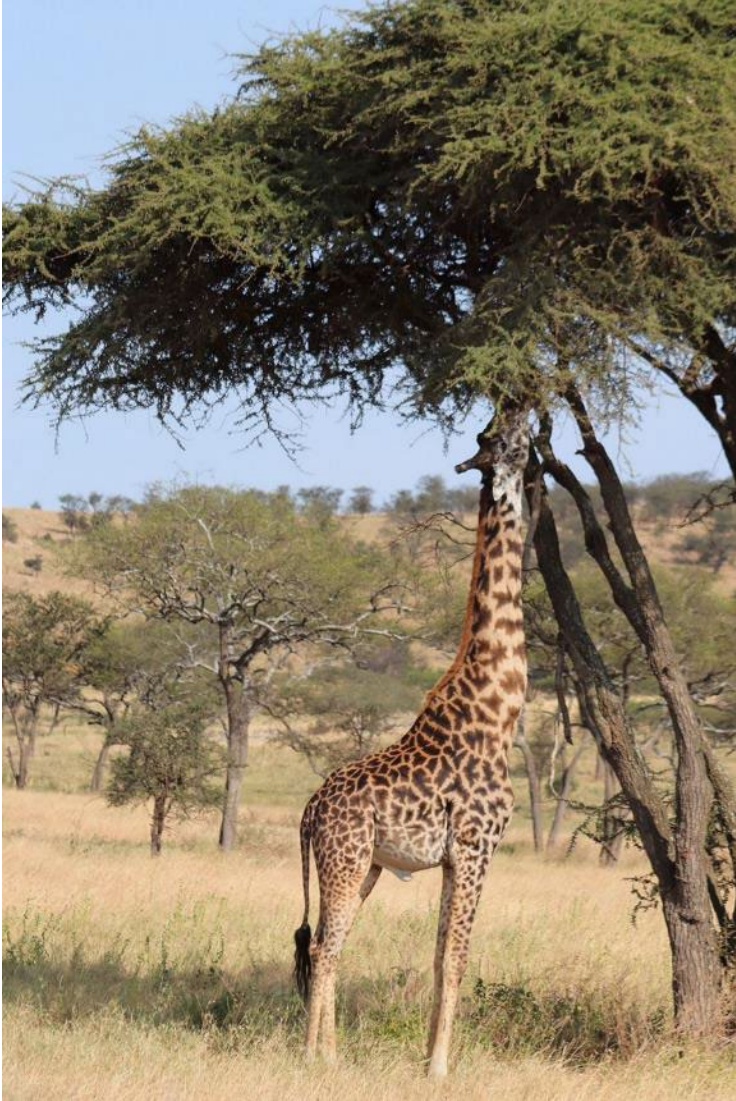
# EPIGENETICS enter the equation $\text{PHENOTYPE} = \text{GENETICS} + \text{ENVIRONMENT}$



# Lamarck's "I told you" moment came a bit too late



Jean-Baptiste Lamarck



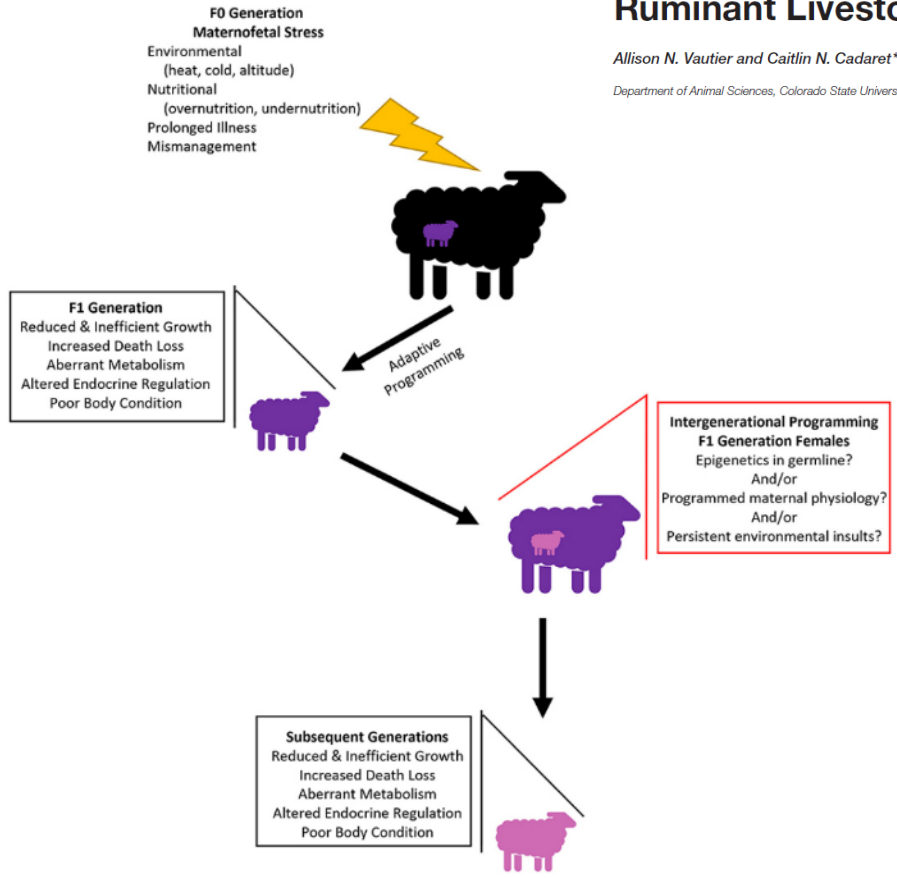
Charles Darwin

# Adaptations can indeed persist through generations



## Long-Term Consequences of Adaptive Fetal Programming in Ruminant Livestock

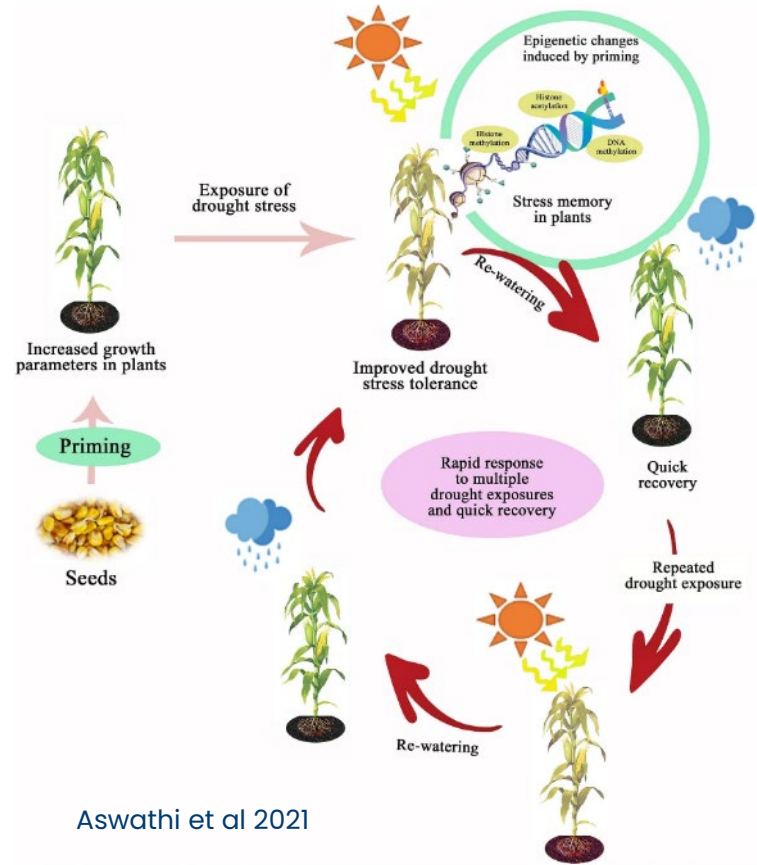
Allison N. Vautier and Caitlin N. Cadaret\*  
 Department of Animal Sciences, Colorado State University, Fort Collins, CO, United States



Vautier and Cadaret 2022

## Seed priming of plants aiding in drought stress tolerance and faster recovery: a review

K. P. Raj Aswathi<sup>1</sup> · Hazem M. Kalaji<sup>2</sup> · Jos T. Puthur<sup>1</sup>



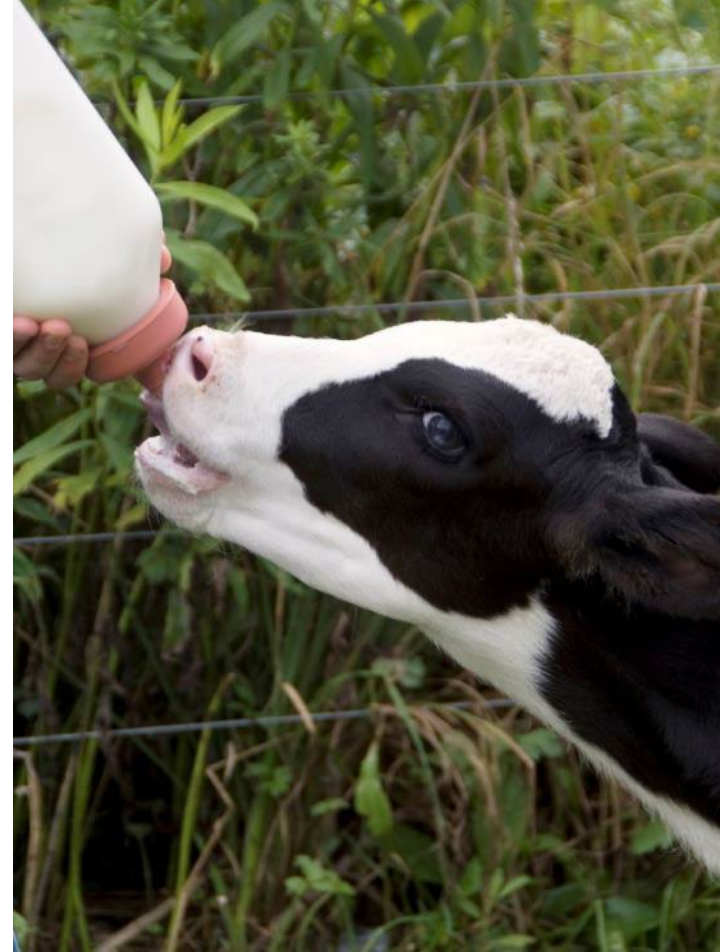
Aswathi et al 2021



# Dairy calves, the perfect thrifty phenotype model



*“Perinatal nutrient deprivation sets adult metabolism for scarcity not for abundance”*



# What are the biological needs of a calf?

## Dairy calves



125l/250l/500l  
Weaning at 6/8/12 weeks

## Biological reference



1.500l  
Weaning at 6 months

- Colostrum
- Health
- Peer and maternal contact
- Ad libitum milk
- Bovine milk composition
- Gradual weaning



Market research\* has shown that **more than 65% of farms** are only feeding 6 litres or less of whole milk or calf milk replacer to their calves.

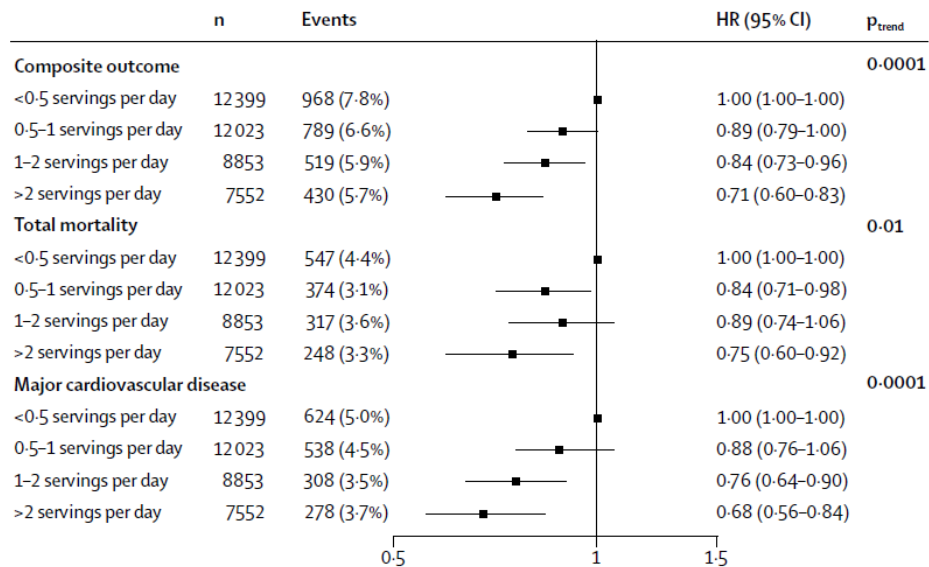
# Milk's effect on global human health



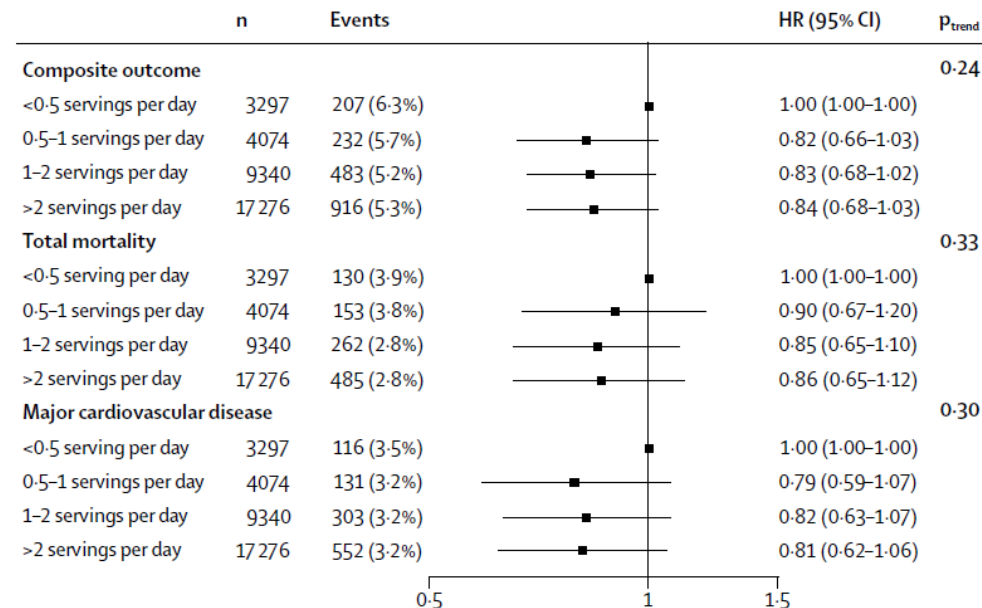
- Prospective cohort study 21 countries from five continents (PURE)
- From 2003 to 2028. 136.000 subjects, 7.000 deaths, 6.000 cardiovascular events



## Consumption of only whole-fat dairy

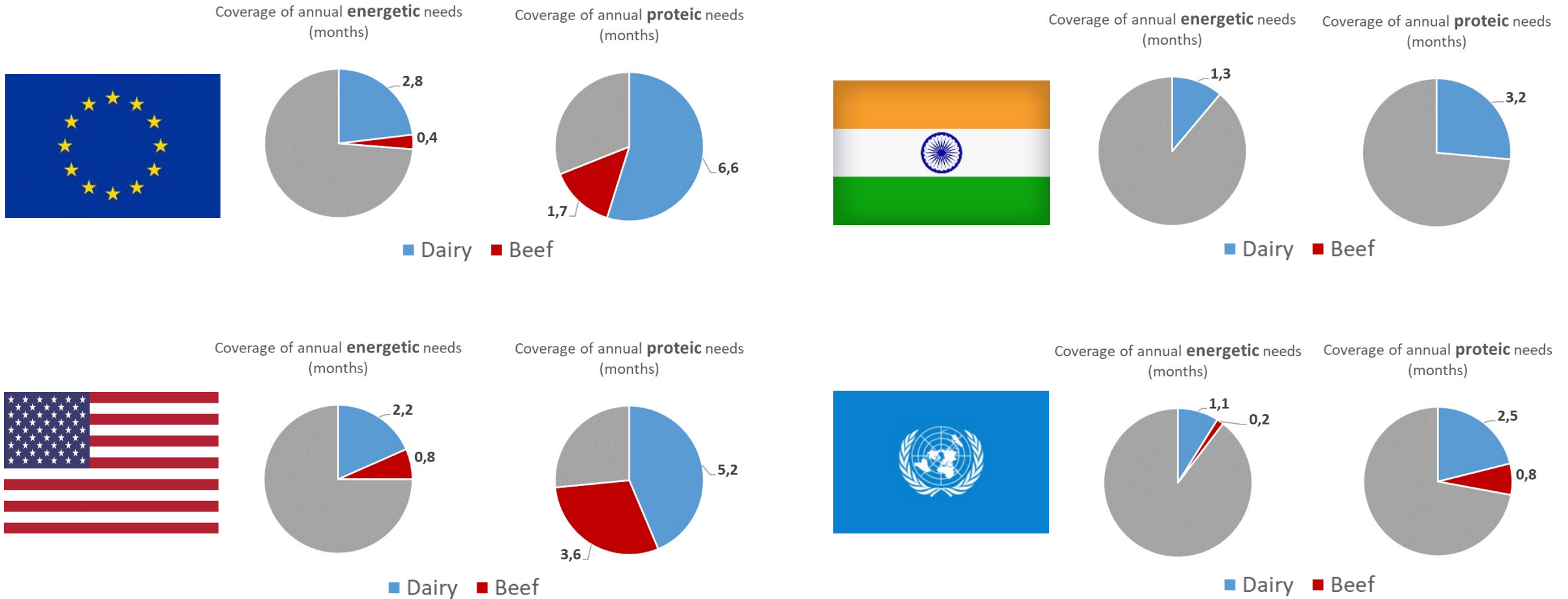


## Consumption of both whole-fat and low-fat dairy



Dehghan et al. The Lancet 2018

# Bovines contribute to our food security



*Milk and beef carcass available estimated by production minus exports. Food available without accounting for food waste. Meat in carcass assumed 65%. Daily calorie requirement assumed 2250kcal and protein requirement 52g.*

# Milk from a nutritional angle



... is the ultimate balanced food



# A complex food in which nothing is left to chance

- Nutrient transfer role
- Signaling role
- Unique composition by species



Table 1. Summary statistics of milk traits

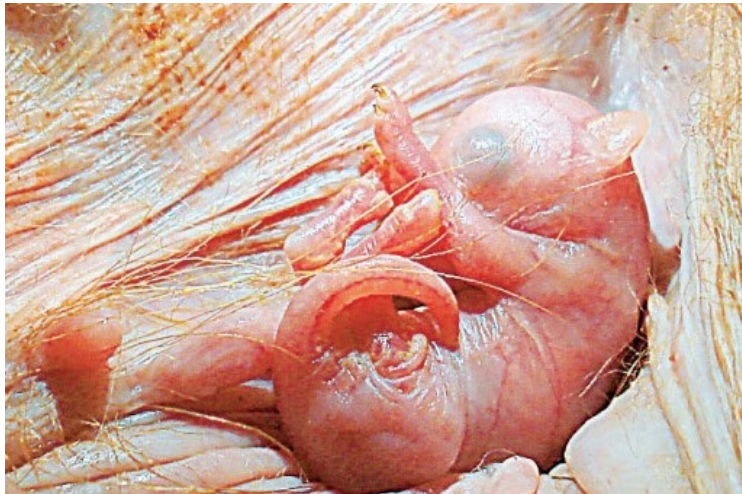
Trait	Mean	Minimum	Maximum	CV, %
Yield, kg/d				
Milk	27.45	3.70	51.00	27.87
Lactose	1.31	0.17	2.61	28.57
Composition, %				
Lactose	4.76	4.06	5.46	3.36
Casein	2.66	1.84	3.53	10.53
Protein	3.38	2.22	4.53	11.24
Fat	4.02	2.10	5.94	15.67
Freezing point, °C	-0.525	-0.552	-0.498	1.90
SCS	2.92	-3.64	10.22	60.27
Mineral content, mg/kg				
Calcium	1,317.00	823.10	1,821.15	12.03
Phosphorus	928.76	600.68	1,258.75	11.48
Magnesium	138.47	62.29	193.87	19.14
Potassium	1,505.24	1,102.00	1,909.11	8.54
Sodium	427.05	273.52	581.59	11.75

Costa et al. 2019

- Much older and evolutionarily conserved than placental reproduction



# Yes, an older reproductive mechanism

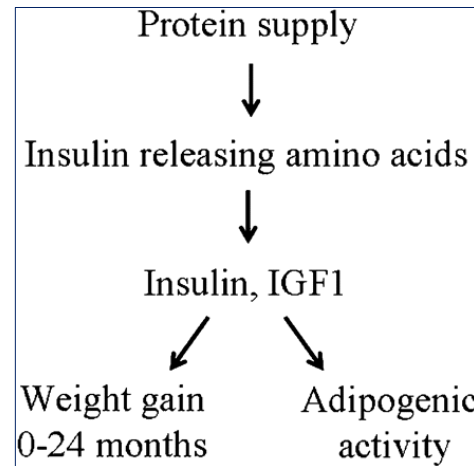


# Nutrient supply seems simple, signaling is not

## Replacing human milk with bovine milk ingredients

CHOP study: Prospective randomized/cohort 990 infants  
2002-2008 nutrient intakes and growth up to age 2  
Health monitored up to age 8

Rapid gain weight from high protein intake is linked with childhood obesity



Koletzko et al. 2009

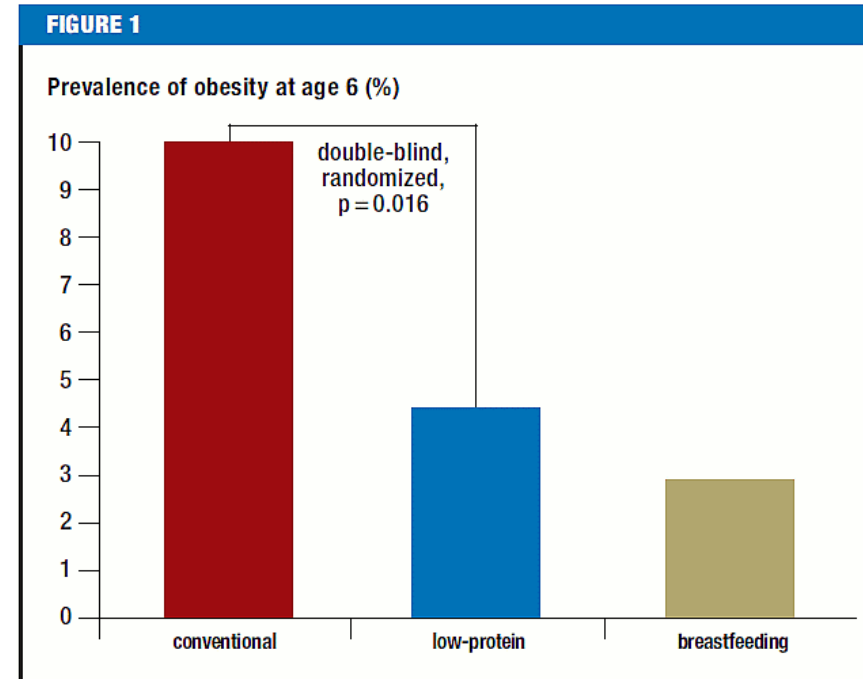


Figure: Ärzteblatt International



# Replacing milk is not just putting nutrients in a liquid feed



- Physicochemical properties
- Digestibility
- Glycaemic signals
- Digestion dynamics
- Barrier function
- Protein quality
- Acid/base metabolism
- Gastrointestinal ecology

## Strong (even causal) evidence of benefits



Onset of puberty <sup>4</sup>

Survival until 1<sup>st</sup> calving <sup>6</sup>

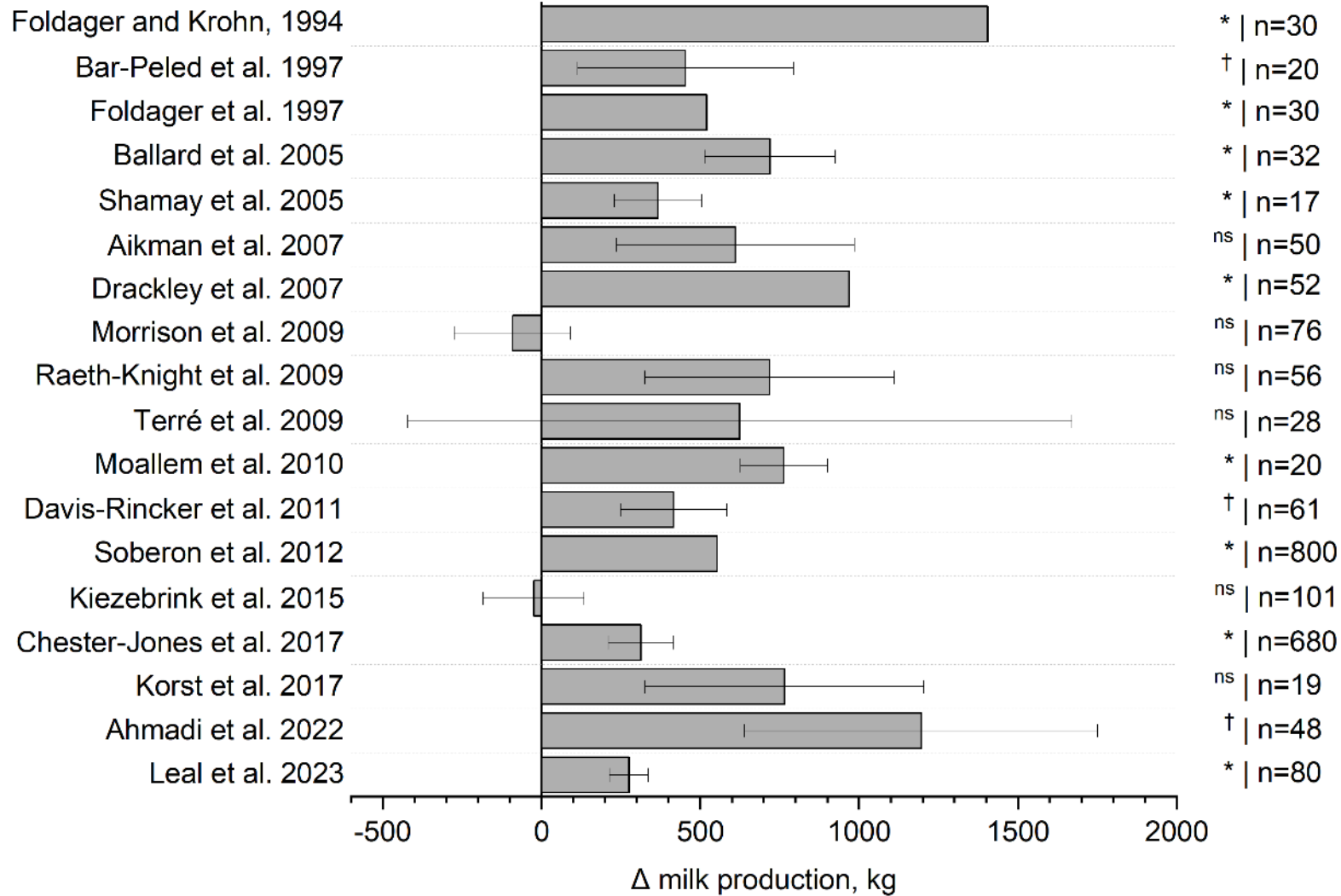
Reduced age at 1<sup>st</sup> calving <sup>1, 3</sup>

Increased milk production <sup>1, 2, 3, 4, 5</sup>

## But how???

<sup>1</sup>Bar-Peled et al. 1998; <sup>2</sup>Drackley et al. 2007; <sup>3</sup>Raeth-Knight et al. 2009  
<sup>4</sup>Davis-Rincker et al. 2011; <sup>5</sup>Soberon et al. 2012; <sup>6</sup>Van de Stroet et al. 2016

# Preweaning growth and 1st lactation milk production



# LifeStart mechanism and metabotype hypothesis



**LIFE START**  
SETS LIFE PERFORMANCE

Grams of daily gain that turn into  
future litres of milk



## How does this happen?

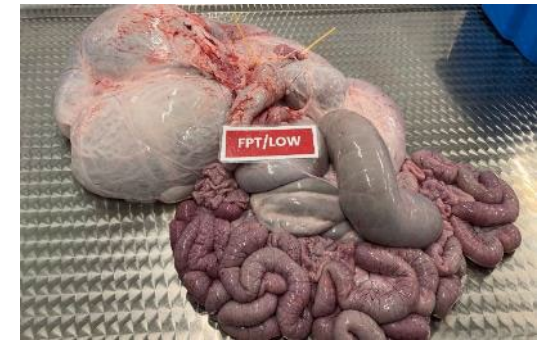
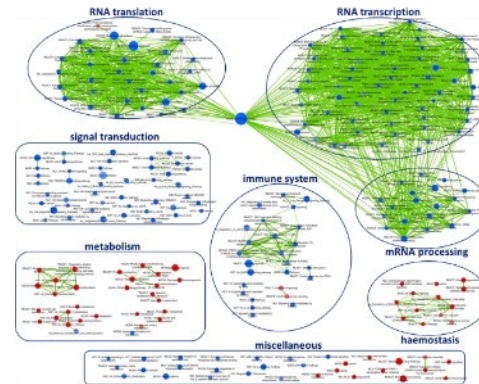
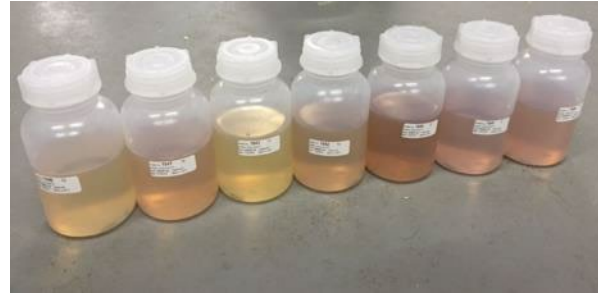
- Simply through development?
- Or really through metabolic programming?

## Nutrients or signals?

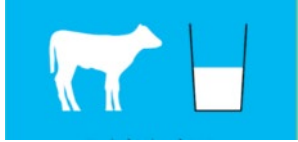
- Nutrient building blocks?
- Signals messaging?

# Research toolset

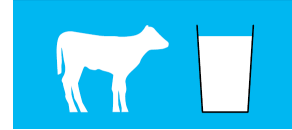
- Nutrient digestibility
- Total nutrient balance
- Transcriptomics
- Metabolomics
- Insulin sensitivity
- Abomasal emptying
- Intestinal permeability
- Respiration chambers
- Histology
- Total body composition



# Enhanced milk supply and organ development



RESTRICTED: 0.6 kg/d MR



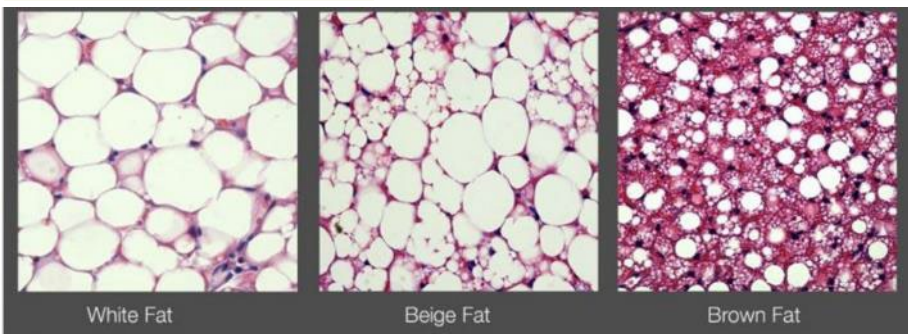
ENHANCED: 1.3 kg/d MR

	Restricted (n=6)	Enhanced (n=6)	P value
Pancreas, g	32.90	29.47	0.61
Pancreas, % of BW	0.06	0.04	0.11
Liver, kg	1.35	2.35	< 0.01
Liver, % of BW	<b>2.23</b>	<b>2.84</b>	<b>&lt; 0.01</b>
Mammary gland, g	75.48	337.58	< 0.01
Parenchyma, g	1.10	6.48	< 0.01
Parenchyma, % of BW	<b>0.002</b>	<b>0.008</b>	<b>&lt; 0.01</b>

(Soberon and Van Amburgh, 2011)

# Change in gene expression profiles

	Changed (P < 0.01)
Mammary	654
Fat	1045
Liver	176
Bone marrow	435
Muscle	651
Pancreas	103



RESEARCH ARTICLE

## Nutrient supply alters transcriptome regulation in adipose tissue of pre-weaning Holstein calves

Leonel N. Leal<sup>1\*</sup>, Josue M. Romao<sup>2\*</sup>, Guido J. Hooiveld<sup>3</sup>, Fernando Soberon<sup>4</sup>, Harma Berends<sup>1</sup>, Mark V. Boekshoten<sup>3</sup>, Michael E. Van Amburgh<sup>5</sup>, Javier Martín-Tereso<sup>1</sup>, Michael A. Steele<sup>2\*</sup>



J. Dairy Sci. 102:1–13  
<https://doi.org/10.3168/jds.2018-15699>

© 2019, The Authors. Published by FASS Inc. and Elsevier Inc. on behalf of the American Dairy Science Association<sup>®</sup>. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### Preweaning nutrient supply alters mammary gland transcriptome expression relating to morphology, lipid accumulation, DNA synthesis, and RNA expression in Holstein heifer calves

K. S. Hare,<sup>1\*</sup> L. N. Leal,<sup>2\*</sup> J. M. Romao,<sup>3</sup> G. J. Hooiveld,<sup>4</sup> F. Soberon,<sup>5</sup> H. Berends,<sup>2</sup> M. E. Van Amburgh,<sup>6</sup> J. Martín-Tereso,<sup>2</sup> and M. A. Steele<sup>1†</sup>

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<sup>2</sup>R&D, Trouw Nutrition, PO Box 299, Amersfoort, 3800 AG, the Netherlands

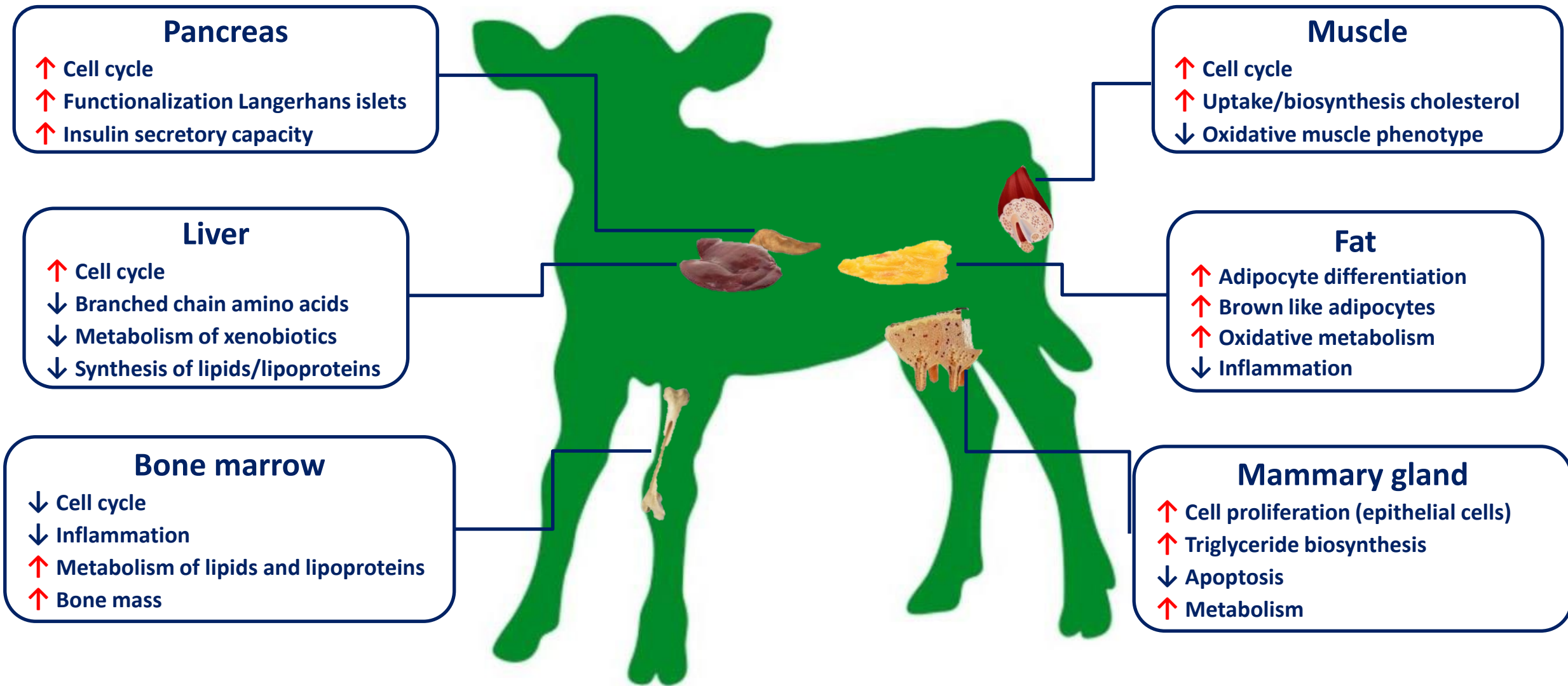
<sup>3</sup>Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Alberta, AB T6G 2P5, Canada

<sup>4</sup>Department of Agrotechnology and Food Sciences, Division of Human Nutrition and Health, Wageningen University, PO Box 17, Wageningen, 6700 AA, the Netherlands

<sup>5</sup>Trouw Nutrition USA, Highland, IL 62249

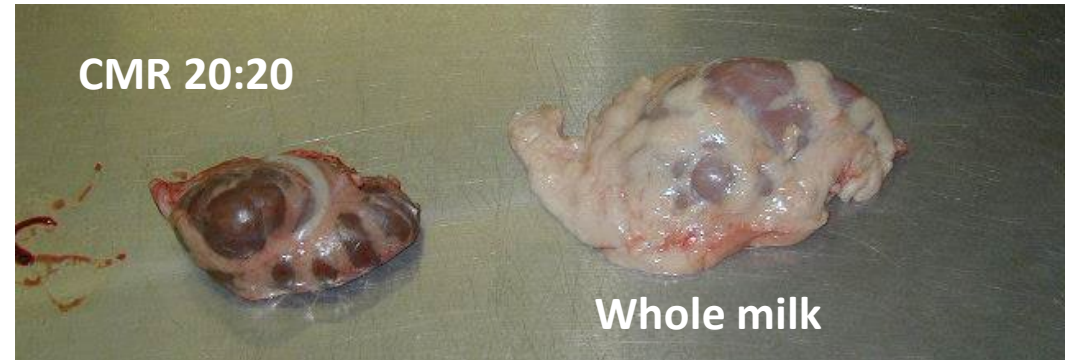
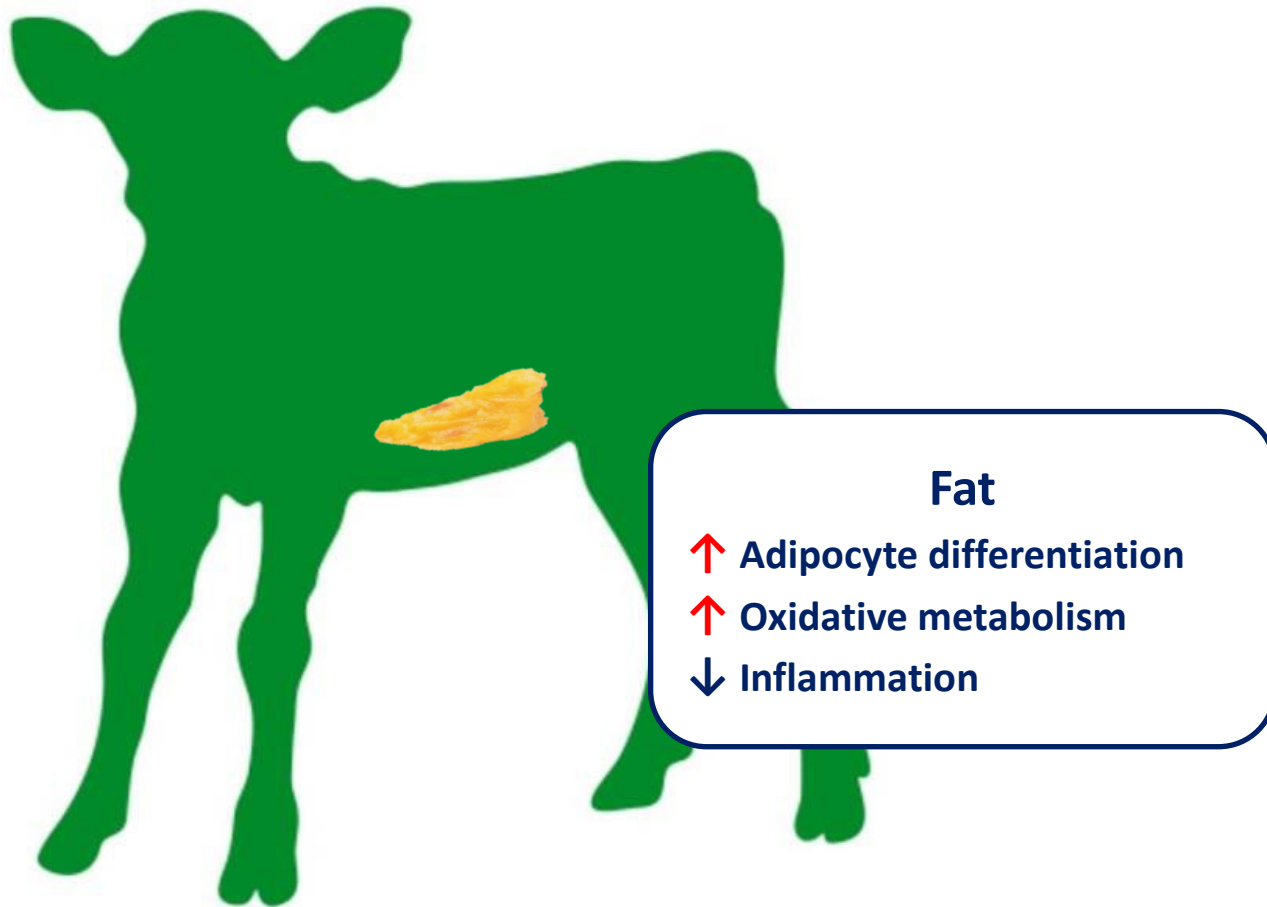
<sup>6</sup>Department of Animal Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14850







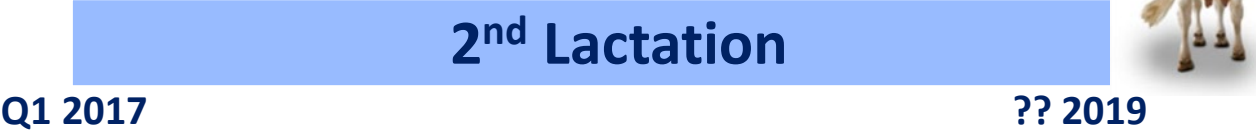
# Lean growth vs lipid dystrophy



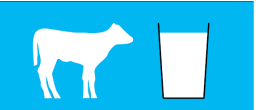
*Van Amburgh, 2011*

- Fat functionality
  - Immune competence
  - Thermoregulation
  - Energy homeostasis

# Longitudinal study at the Trouw Nutrition Dairy facility (NL)



Conventional: 0.6 kg/d

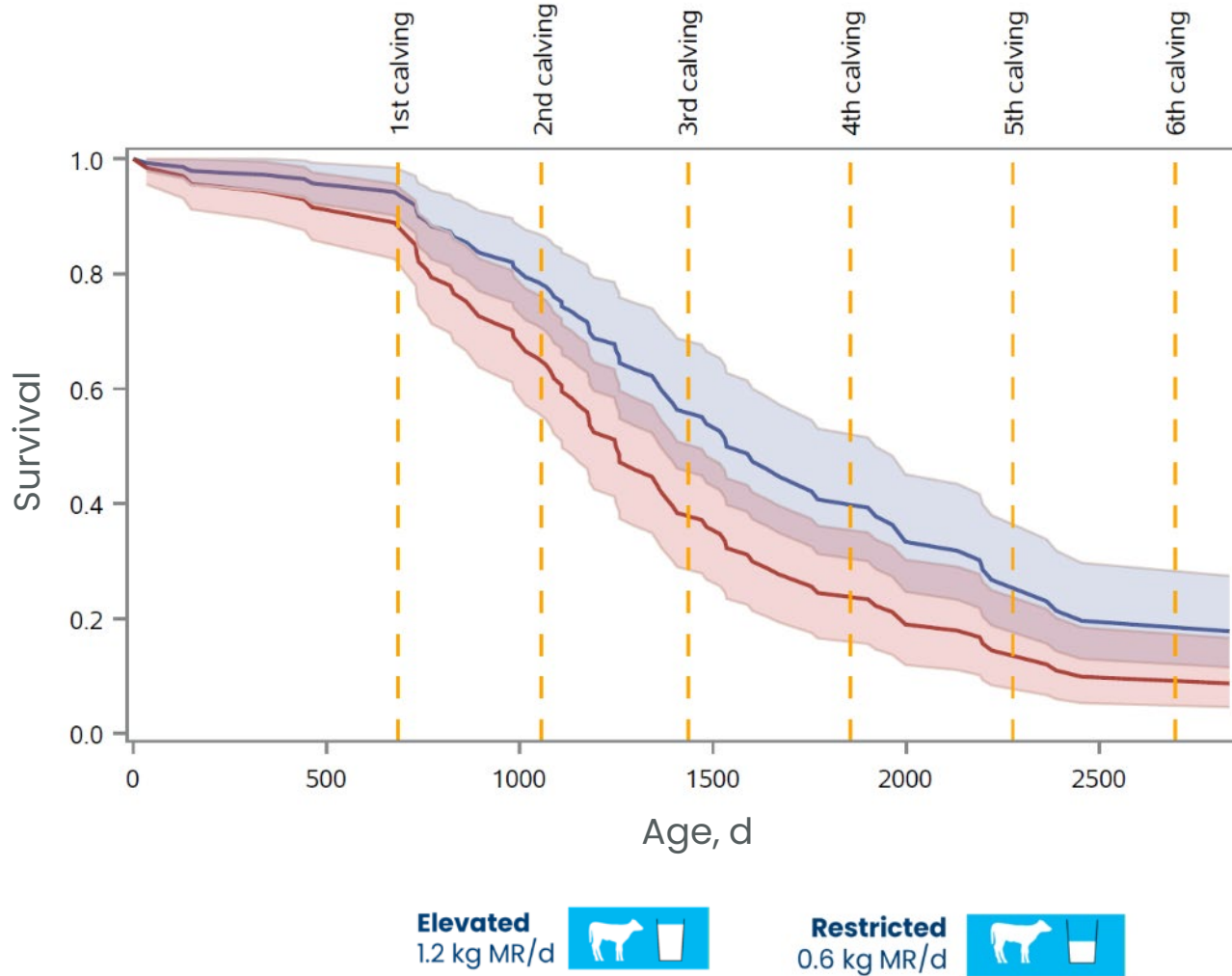


LifeStart: 1.2 kg/d

# Preweaning plane on 1<sup>st</sup> and 2<sup>nd</sup> lactation

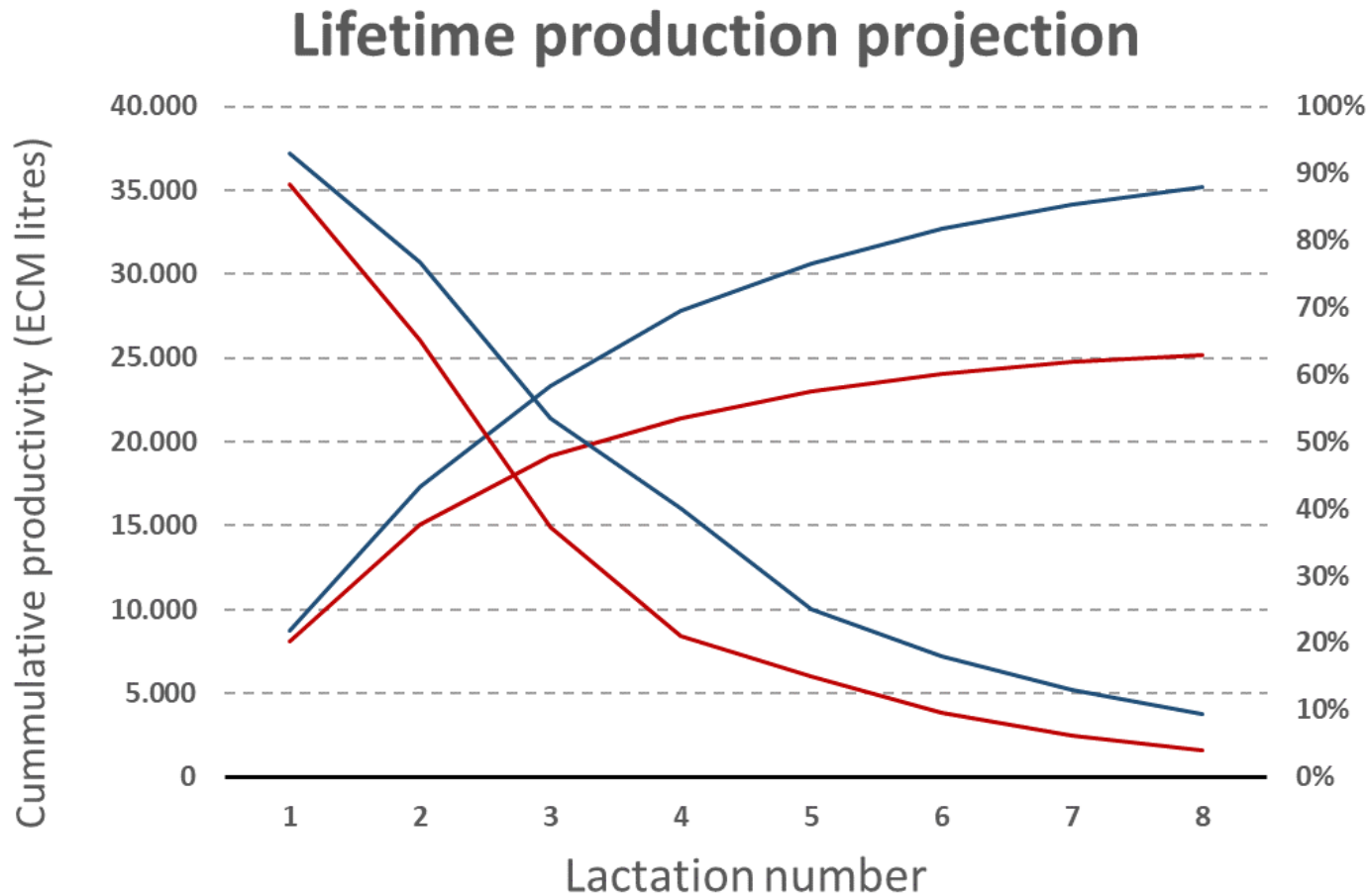
Item	Treatment		SEM	P-value	
	ELE	RES		Treat	Treat x WIM
<b>First lactation (n=64 cows)</b>					
Dry-matter intake, kg/d	19.7	19.0	0.2	<b>0.01</b>	0.62
Milk yield, kg/d	29.1	29.1	0.4	0.98	0.25
Fat-protein corrected milk, kg/d	30.8	29.9	0.2	<b>0.01</b>	0.91
Milk composition					
Fat, g/d	1296	1213	9	<b>&lt;0.01</b>	0.87
Protein, g/d	995	996	10	0.95	0.73
Lactose, g/d	1363	1368	18	0.86	0.96
Body weight, kg	585	593	7	0.44	0.96
Body condition score, 1-5 scale	3.21	3.30	0.02	<b>&lt;0.01</b>	0.46
<b>Second lactation (n=45 cows)</b>					
Dry-matter intake, kg/d	23.3	23.0	0.3	0.50	0.94
Milk yield, kg/d	34.7	33.3	0.7	0.23	0.12
Fat-protein corrected milk, kg/d	36.8	35.5	0.5	0.12	0.38
Milk composition					
Fat, g/d	1536	1464	22	<b>0.04</b>	0.73
Protein, g/d	1208	1184	16	0.33	<b>0.05</b>
Lactose, g/d	1593	1545	32	0.35	0.23
Body weight, kg	649	665	8	0.20	1.00
Body condition score, 1-5 scale	3.04	3.17	0.04	0.08	0.68

# Preweaning plane on survival



Item	Treatment		<i>P-value</i>
	ELE (n = 43)	RES (n = 43)	
<b>Survival 1<sup>st</sup> calving</b> % of total (n calving)	93% (40)	88% (38)	0.36
<b>Survival 2<sup>nd</sup> calving</b> % of total (n calving)	77% (33)	65% (28)	<b>0.07</b>
<b>Survival 3<sup>rd</sup> calving</b> % of total (n calving)	54% (23)	37% (16)	<b>0.05</b>
<b>Survival 4<sup>th</sup> calving</b> % of total (n calving)	42% (18)	23% (10)	<b>0.02</b>
<b>Survival 5<sup>th</sup> calving</b> % of total (n calving)	26% (11)	14% (6)	<b>0.02</b>
<b>Survival 6<sup>th</sup> calving</b> % of total (n calving)	21% (9)	7% (3)	<b>0.02</b>
<b>Survival August 2023</b> % of total (n calving)	21% (9)	5% (2)	<b>0.02</b>

# Cumulative production corrected by culling



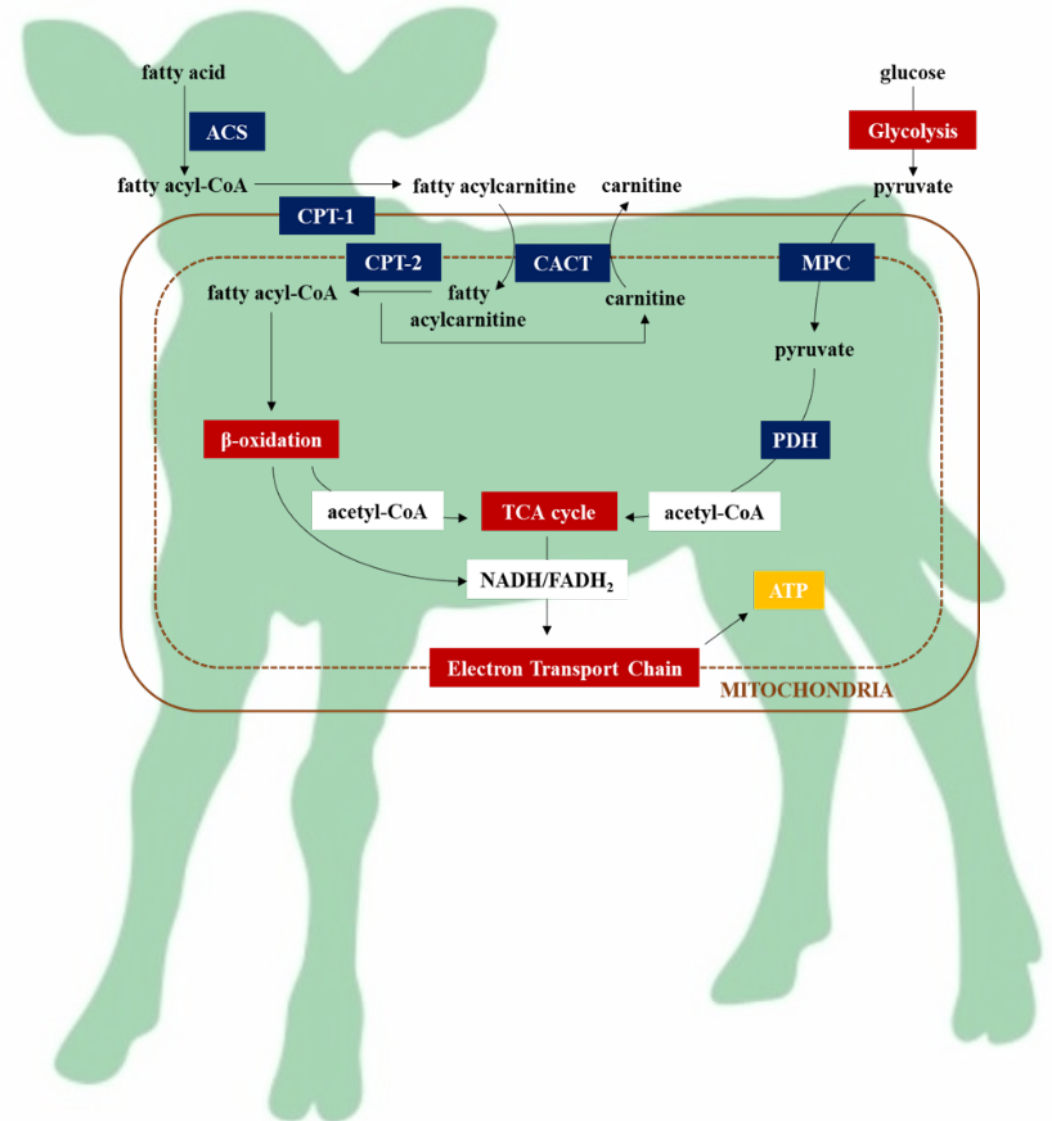
35.000 l in 3.3 lactations  
10,600l/lactation  
vs  
25.000l in 2.5 lactations  
10,000/lactation



- More milk to sell
- Fewer heifers to raise

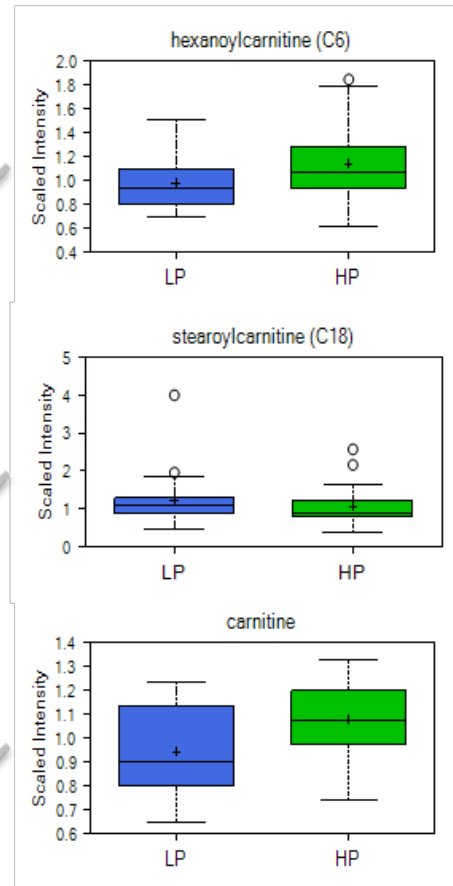
# Energy metabolism of the calf

	Biochemical	ELE d2 vs. RES d2	ELE d49 vs. RES d49
Fatty Acid Metabolism	butyrylcarnitine	1.14	0.89
	butyrylglycine	1.08	0.42
	propionylcarnitine	1.06	0.57
	propionylglycine	1.02	0.24
	valerylglycine	0.97	0.14
	hexanoylglycine	0.97	0.70
	N-palmitoylglycine	1.06	1.10
	acetylcarnitine	1.10	1.28
	3-hydroxybutyrylcarnitine	1.14	1.16
	hexanoylcarnitine	1.13	1.22
	octanoylcarnitine	1.05	1.41
	decanoylcarnitine	1.06	1.49
	laurylcarnitine	1.01	1.37
	myristoylcarnitine	1.06	1.25
	palmitoylcarnitine	1.07	1.61
	palmitoleoylcarnitine	1.05	1.24
	stearoylcarnitine	1.08	1.23
	linoleoylcarnitine	0.90	1.29
	oleoylcarnitine	1.05	1.26
	myristoleoylcarnitine	1.14	1.52
suberoylcarnitine	0.83	0.94	
adipoylcarnitine	0.89	1.18	
carnitine	1.04	1.20	
3-hydroxybutyrate (BHBA)	1.09	0.39	
TCA Cycle	citrate	0.95	0.80
	aconitate [cis or trans]	0.92	0.86
	isocitrate	0.98	0.91
	alpha-ketoglutarate	1.08	0.91
	succinylcarnitine	0.98	1.27
	succinate	1.08	0.85
	fumarate	1.09	0.83
	malate	1.11	0.79
	tricarballylate	0.83	0.12
	2-methylcitrate/homocitrate	0.94	0.62



# Increased $\beta$ -oxidation capacity in LifeStart heifers

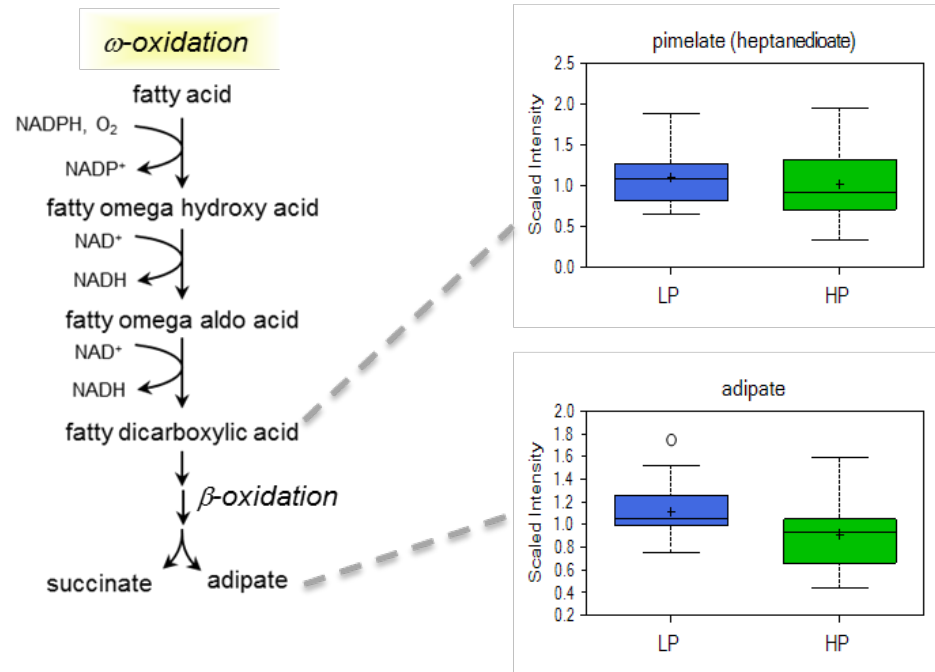
Pathway	Biochemical	HP LP
Fatty Acid Metabolism (Acylcarnitine)	acetylcarnitine (C2)	1.12
	3-hydroxybutyrylcarnitine (1)	1.09
	3-hydroxybutyrylcarnitine (2)	1.03
	hexanoylcarnitine (C6)	1.17
	octanoylcarnitine (C8)	1.16
	decanoylcarnitine (C10)	0.97
	cis-4-decenoylcarnitine (C10:1)	0.99
	laurylcarnitine (C12)	0.82
	myristoylcarnitine (C14)	0.97
	palmitoylcarnitine (C16)	0.88
	stearoylcarnitine (C18)	0.87
	oleoylcarnitine (C18:1)	0.88
	myristoleoylcarnitine (C14:1)*	0.84
	suberoylcarnitine (C8-DC)	0.81
	adipoylcarnitine (C6-DC)	0.97
Carnitine Metabolism	deoxycarnitine	1.07
	carnitine	1.14



- $\beta$ -oxidation is key for energy production (Krebs cycle)
- Increased capacity for  $\beta$ -oxidation in LS heifers
  - higher carnitine
  - lower long chain acylcarnitines
  - Increase in short and medium chain acylcarnitines

# Reduced $\omega$ -oxidation in Enhanced heifers

Biochemical	HP LP
dimethylmalonic acid	0.87
3-methylglutarate/2-methylglutarate	0.89
2-hydroxyglutarate	0.88
adipate	0.82
3-carboxyadipate	0.85
2-hydroxyadipate	0.9
3-methyladipate	0.92
maleate	0.9
pimelate (heptanedioate)	0.92
suberate (octanedioate)	0.95
azelate (nonanedioate)	0.98
sebacate (decanedioate)	0.99
undecanedioate	0.97
dodecanedioate	0.94
hexadecanedioate	0.96
octadecanedioate	0.98
eicosanodioate	0.91
docosanodioate	0.94



- **Fatty acids can be also oxidized through  $\omega$ -oxidation**
- **Occurs mainly**
  - mitochondria are overwhelmed
  - $\beta$ -oxidation is impaired
- **Lower  $\omega$ -oxidation in ENH heifers**
  - low adipate and other dicarboxylic fatty acids

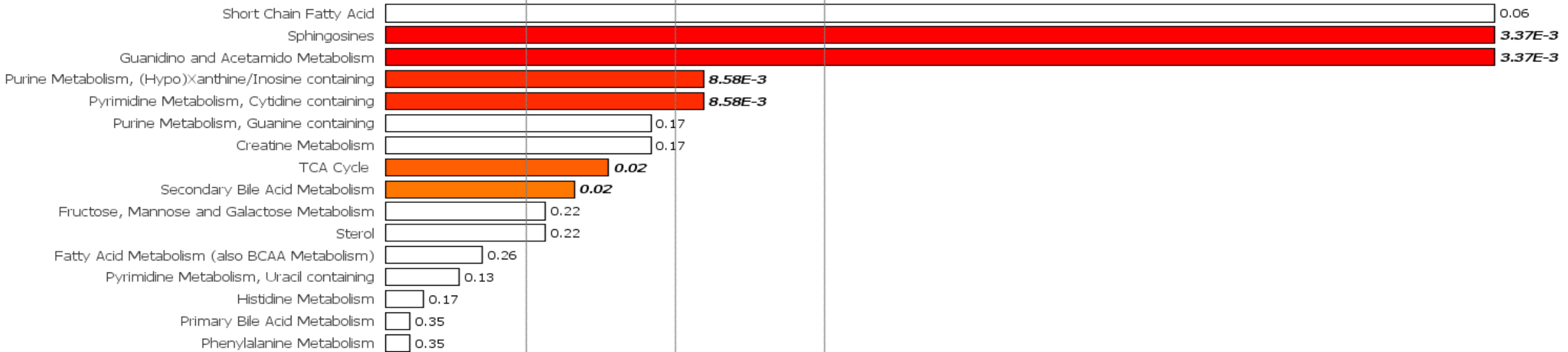


# Different metabotypes at 60 DIM....

HP / / LP

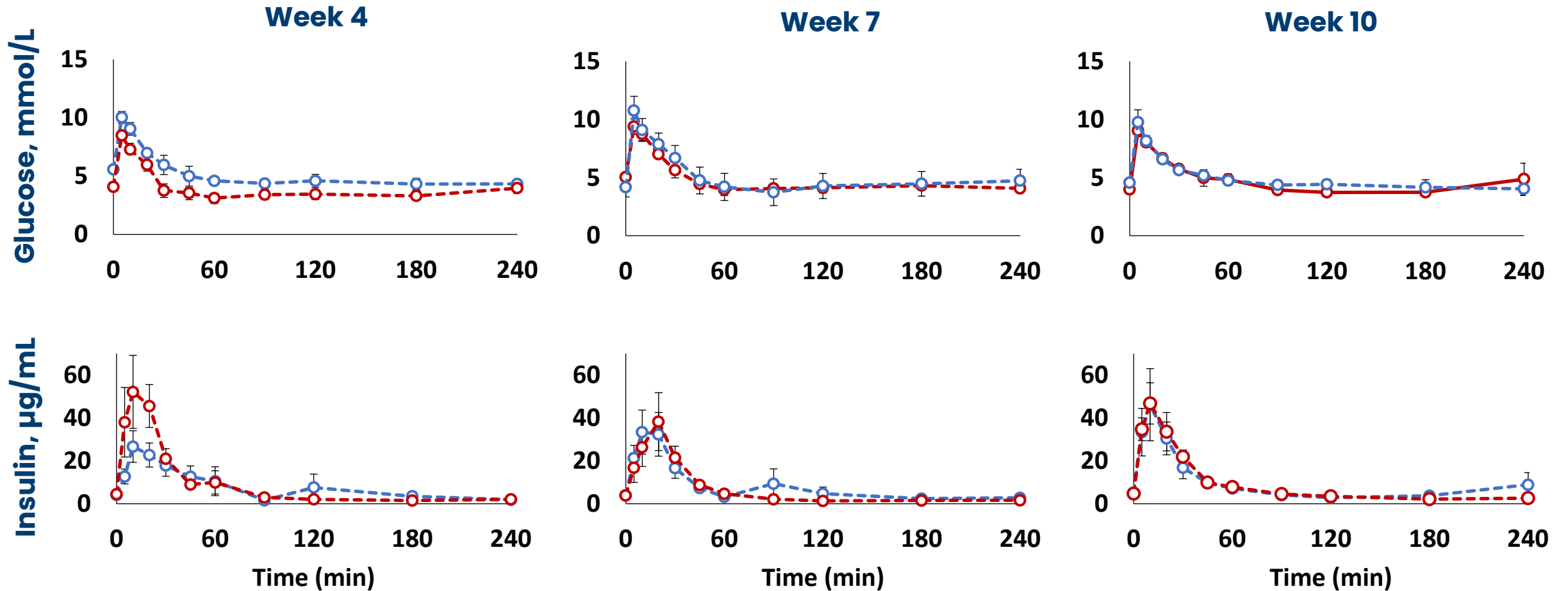
Fold Enrichment

3 4 5 6 7 8 9 10 11 12 13 14 15 16 17



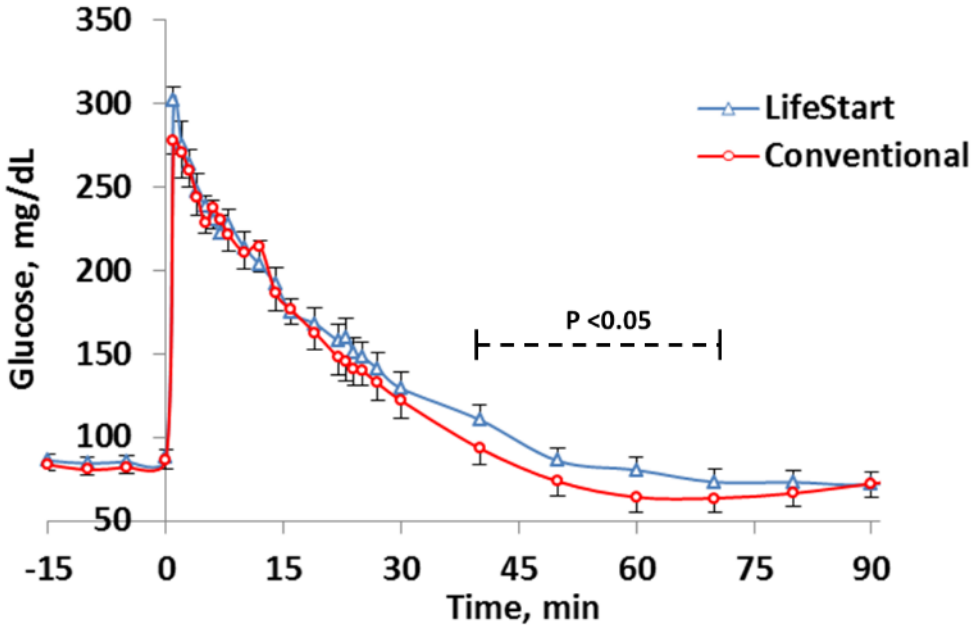
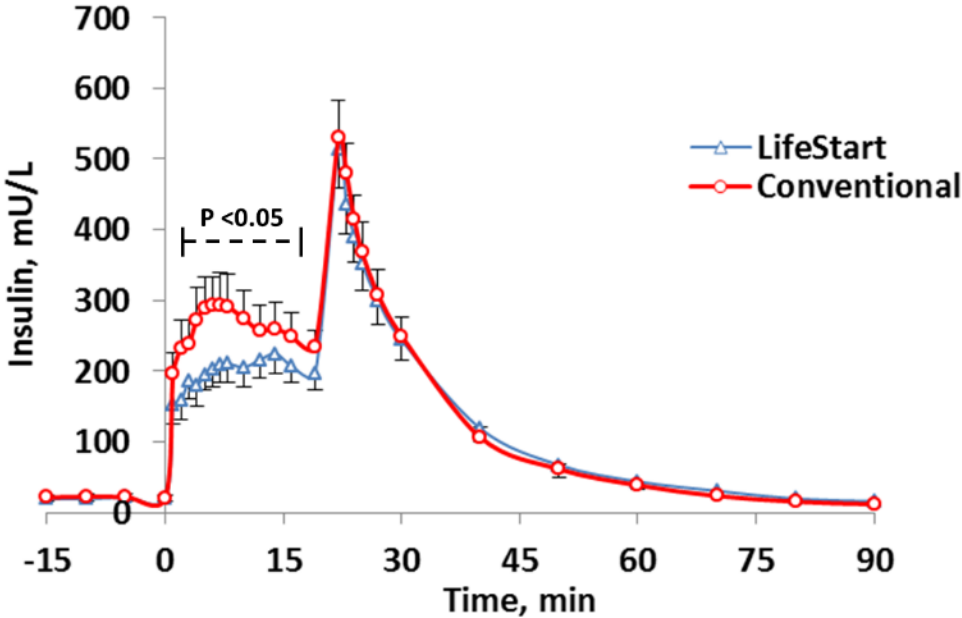
...adaptations are still present in 1<sup>st</sup> lactation (metabolic programming)...

# No effect on glucose tolerance at wk 4, 7 or 10

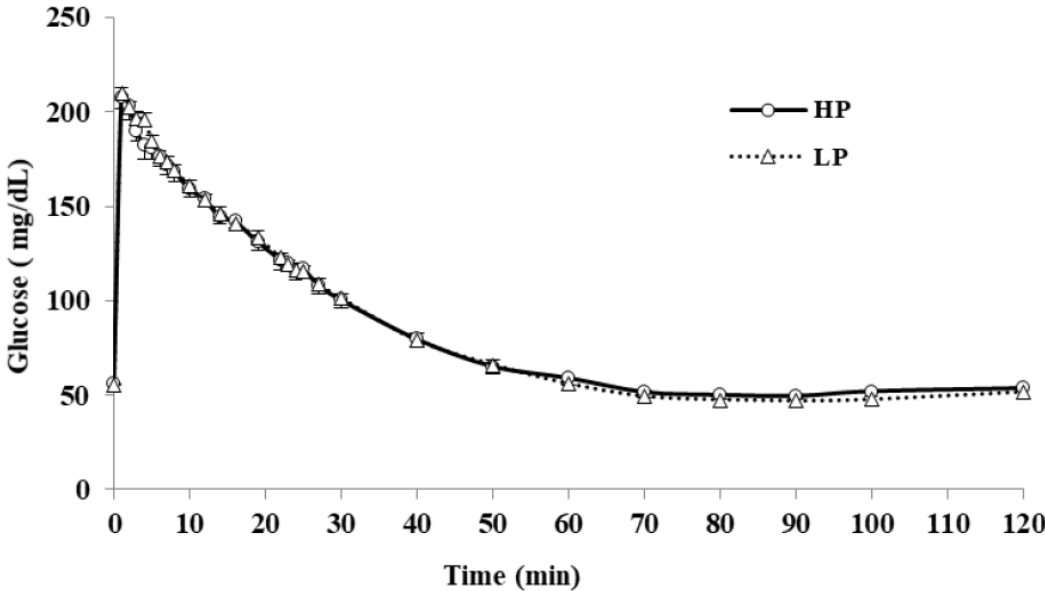
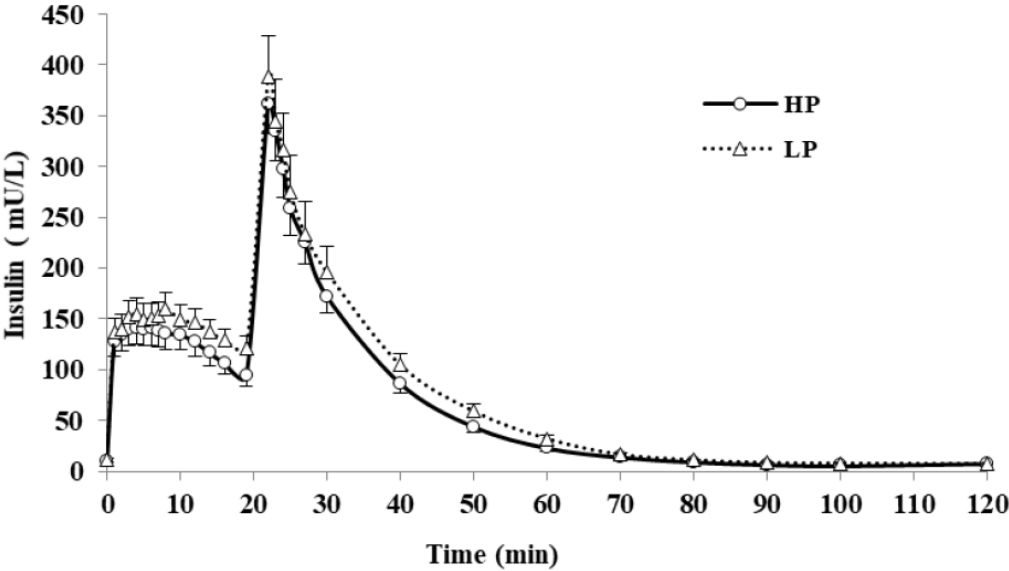


— Elevated 1.2 kg MR/d  — Restricted 0.6 kg MR/d 

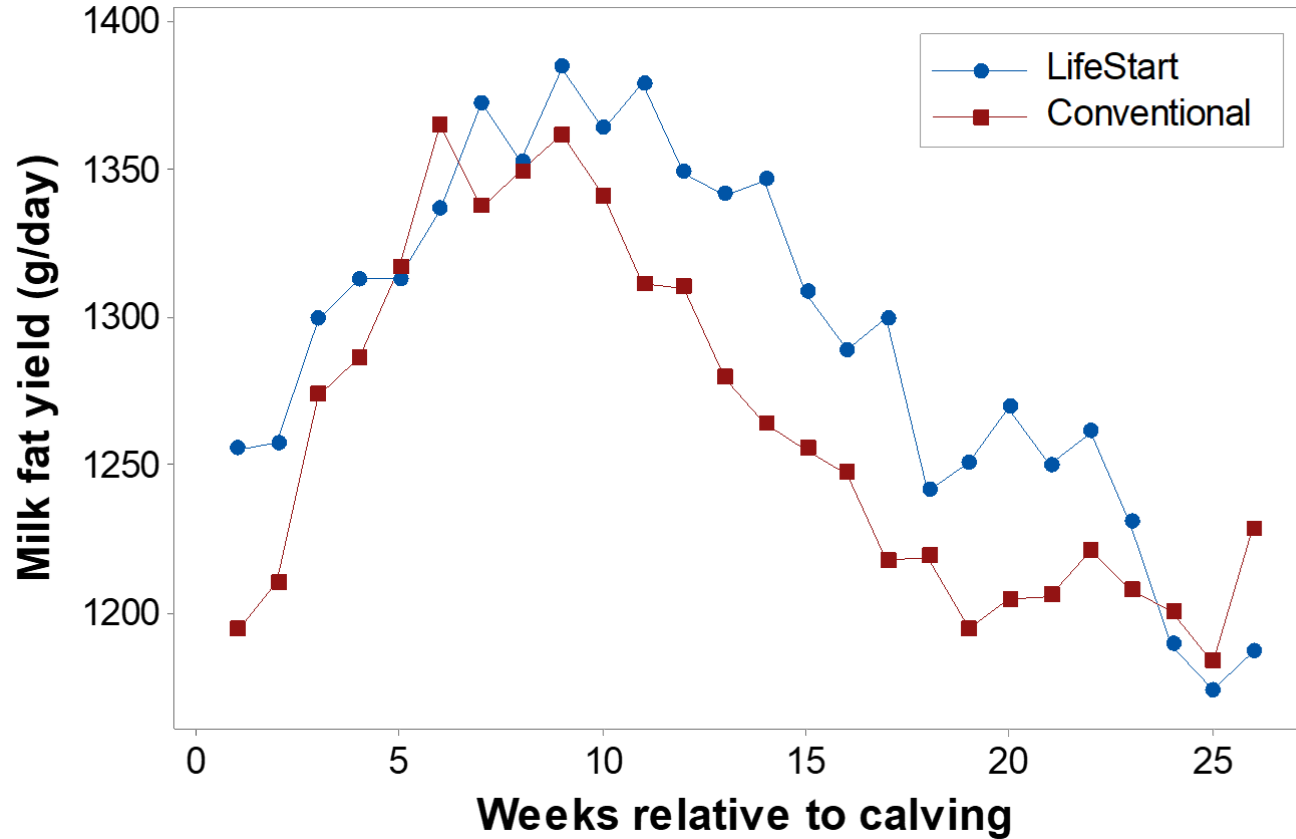
# Glucose response heifers



# Glucose response cows (lactation)

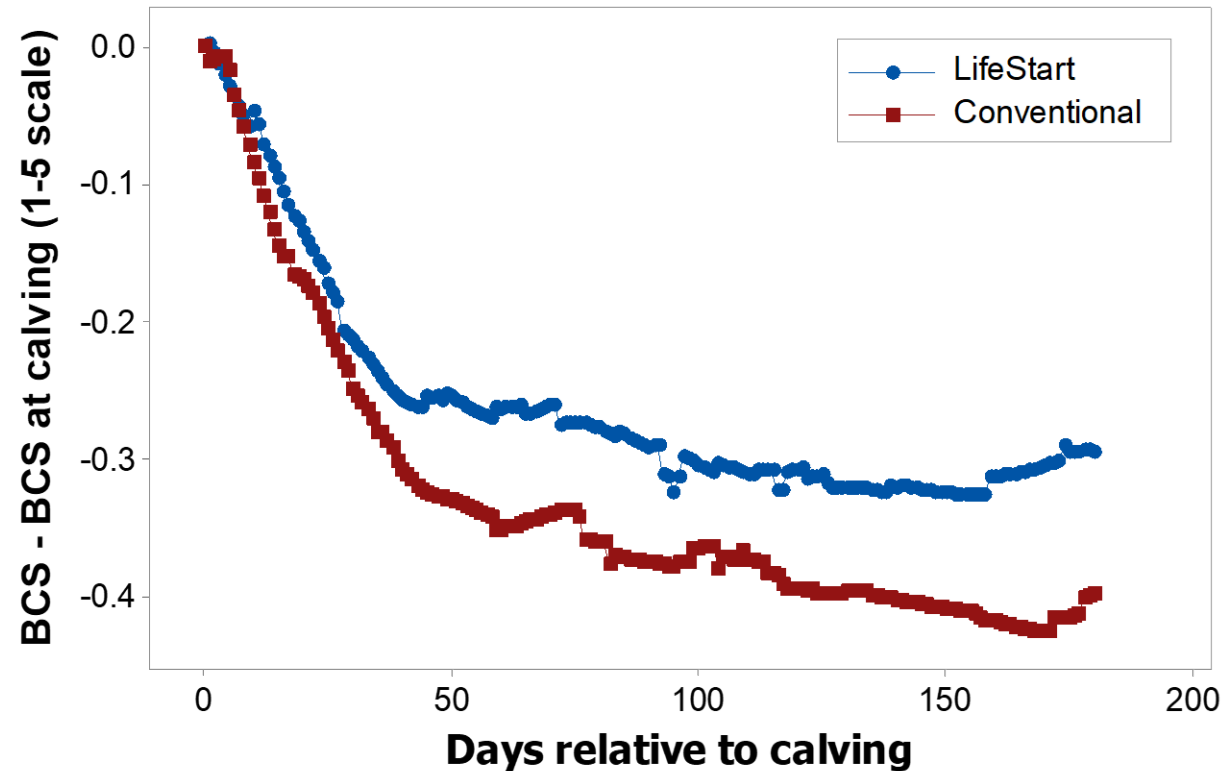


# Phenotypically, milk fat yield is higher



However...

# Change in body condition score is also higher



... so not at the expense of body reserves...

# Conclusions from calf phase

- **Clear dietary differences between the 2 treatments at d49**
  - >45% of all metabolites
- **Metabolic pathways are affected by the total amount of nutrients supplied and by the nature of the diet**
  - ketogenic vs. glucogenic
- **Dietary interventions can lead to profound metabolic adaptations**
  - energy metabolism
  - organ development
  - microbiome



**BIG CALF vs. SMALL CALF**

# Conclusions from heifer phase

- Differences in age at 1<sup>st</sup> AI can be explained by BW
- LifeStart heifers got pregnant earlier than Conventional heifers
- Preweaning feeding may have an effect on glucose/insulin metabolism at 12 months (FSIGTT)
- Metabolomics indicate that pre-weaning plane of nutrition can have carry over effects
  - energy metabolism ( $\beta$ -oxidation vs.  $\omega$ -oxidation)
  - amino acid metabolism
  - microbial fermentation end-products





# Conclusions from lactation

- **Metabolism**
  - Energy metabolism
  - Microbially derived metabolites
  - Protein metabolism
  - Insulin/glucose metabolism
- **Breeding**
  - Earlier 1<sup>st</sup> AI
  - Earlier pregnancy
  - Earlier 1<sup>st</sup> calving
- **Lactation (until 180 DIM)**
  - Greater fat yield and FCM
  - Lower BCS mobilization
  - Improved survival



## Traditional

Intake 10% BW  
ADG 500-600 g/d

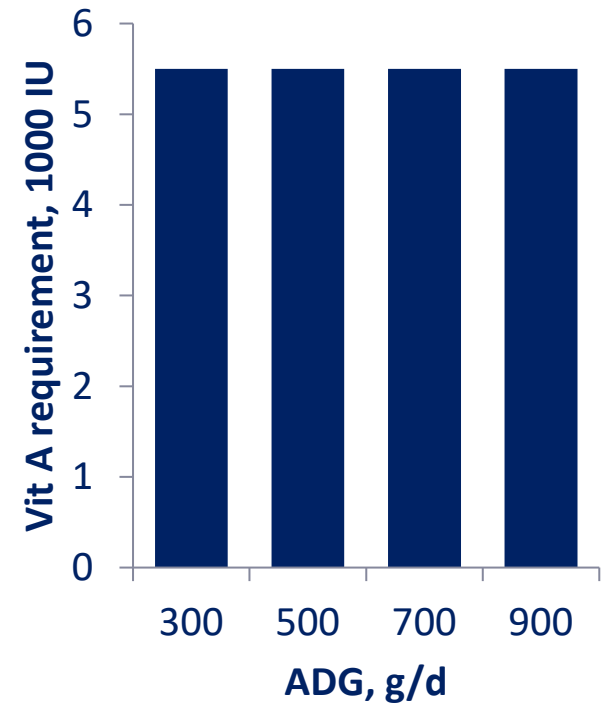
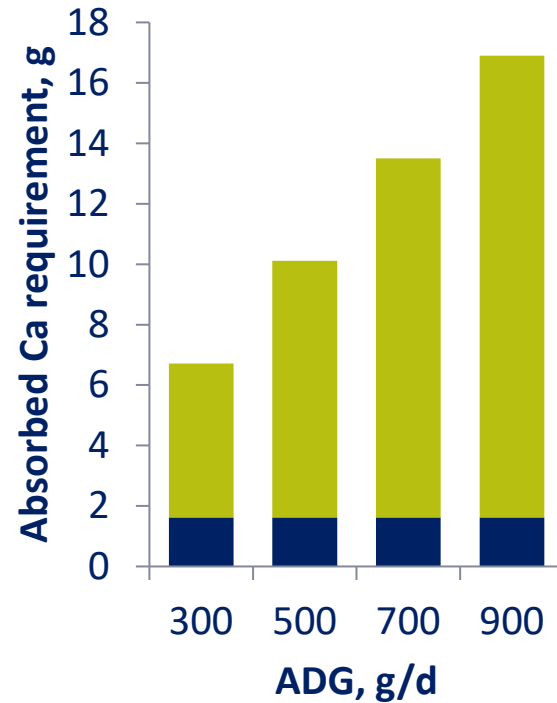
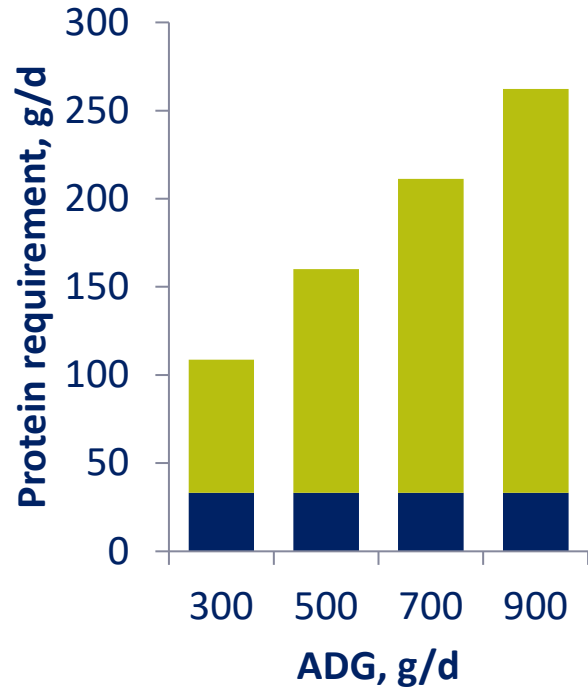
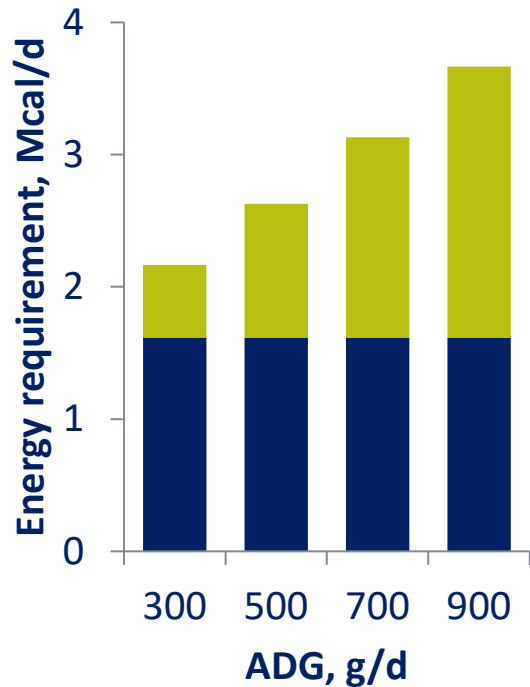


## LIFESTART®

SETS LIFE PERFORMANCE

Intake 20% BW  
ADG 800-1000 g/d

■ Growth  
■ Maintenance



# Restoring nutrient supply and signaling

Biological reference



2015

Increasing the fat inclusion

- Increased survival in preweaning phase
- Improvements of fecal scores / therapeutical treatments
- Hormonal homeostasis (insulin-glucose metabolism)

Fat sources in milk replacer

- Possible to formulate based on FA profile
- High PUFA negative for calves
- Inclusion of dairy cream largely beneficial

Balancing FA

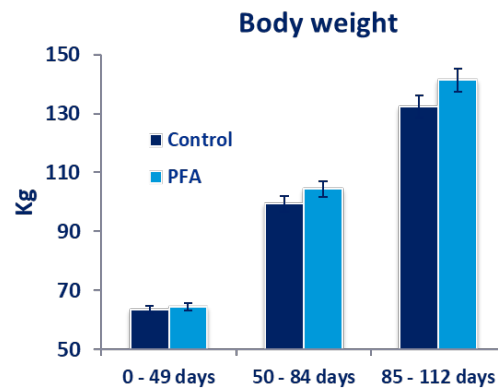
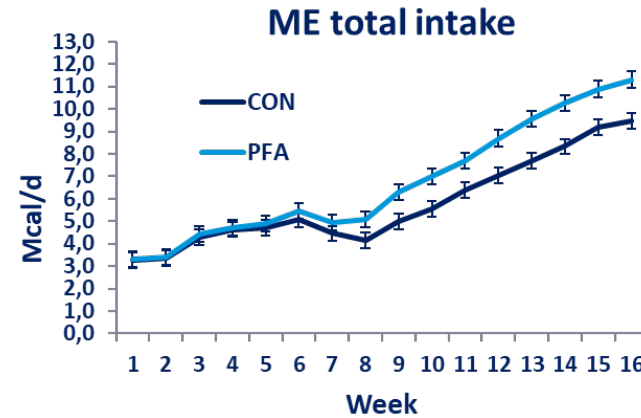
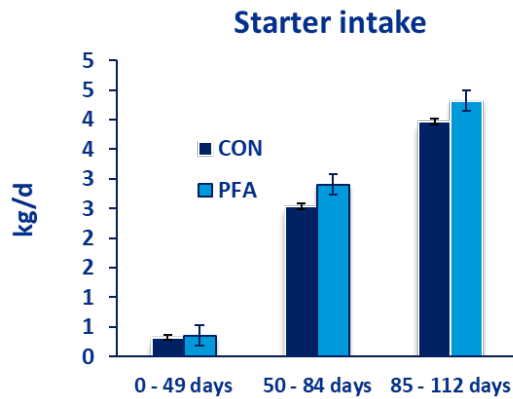
- Improvement in fat digestibility and fecal scores
- Increased MR and starter intakes → increased performance
- Enhanced rumen papillae / ileum villi development

2023

# Restoring early weaning nutrient shortages

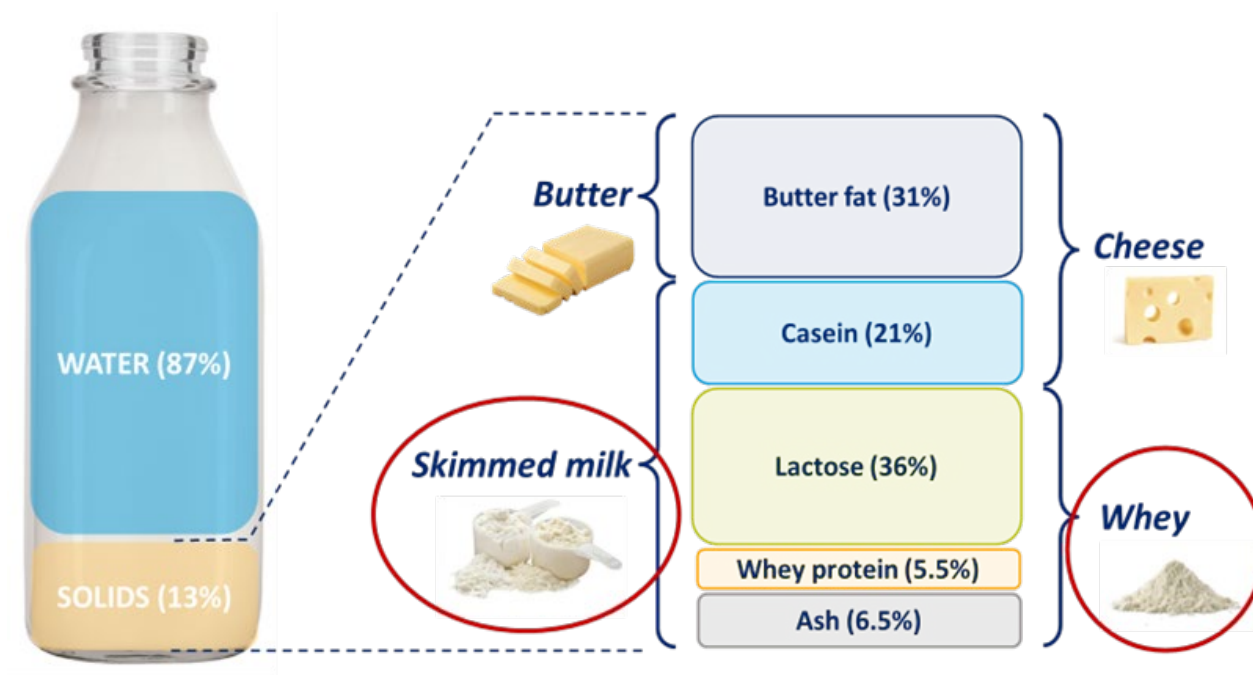
Adding fat to starters without compromising rumen development and function results in greater energy intake and growth

Biological reference



# Restoring milk quality

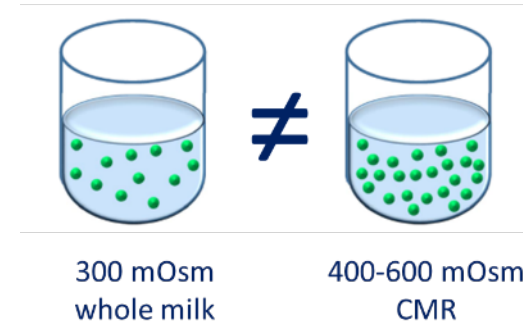
When replacing milk...  
...nothing can be left to chance...



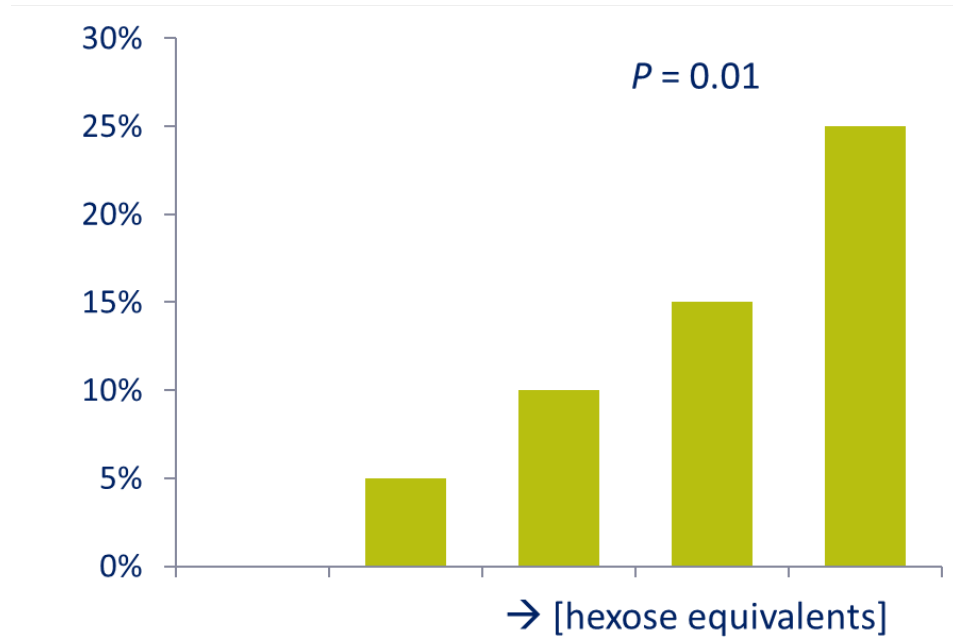
Wilms, J. Trouw Nutrition R&D

- Water
- Physicochemical composition
- Carbohydrate fraction
- Fat fraction
- Protein fraction
- Functional components
- Minerals
- Vitamins

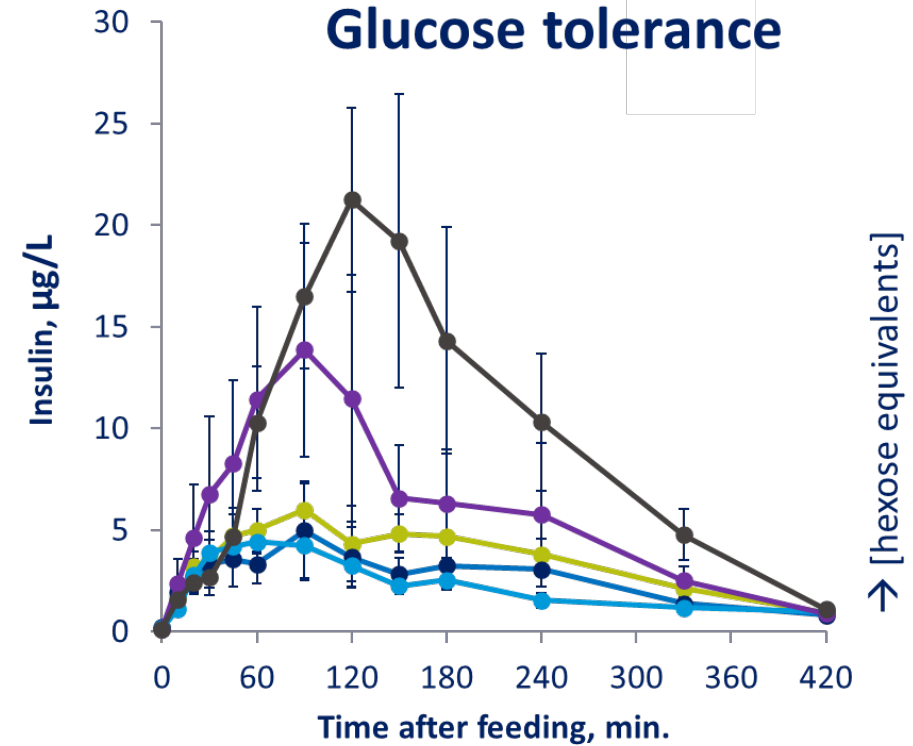
# Osmolality as an example



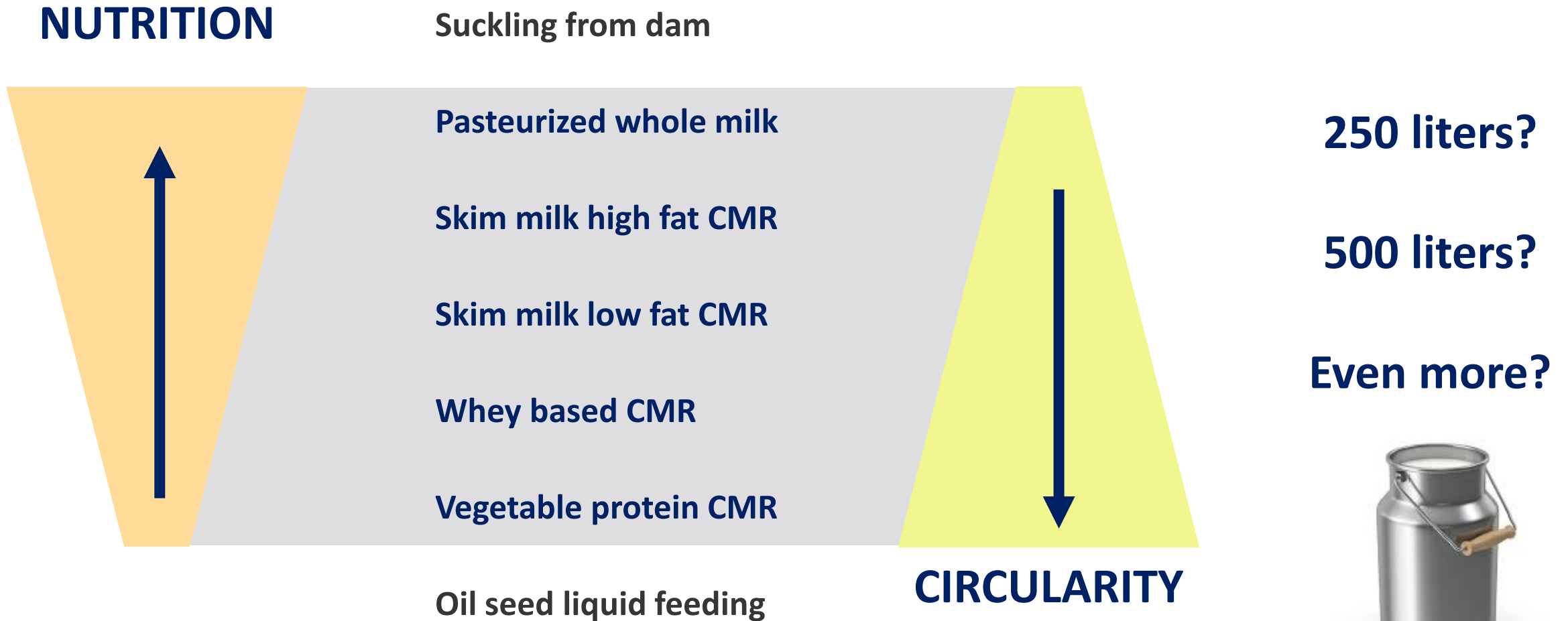
## Mortality



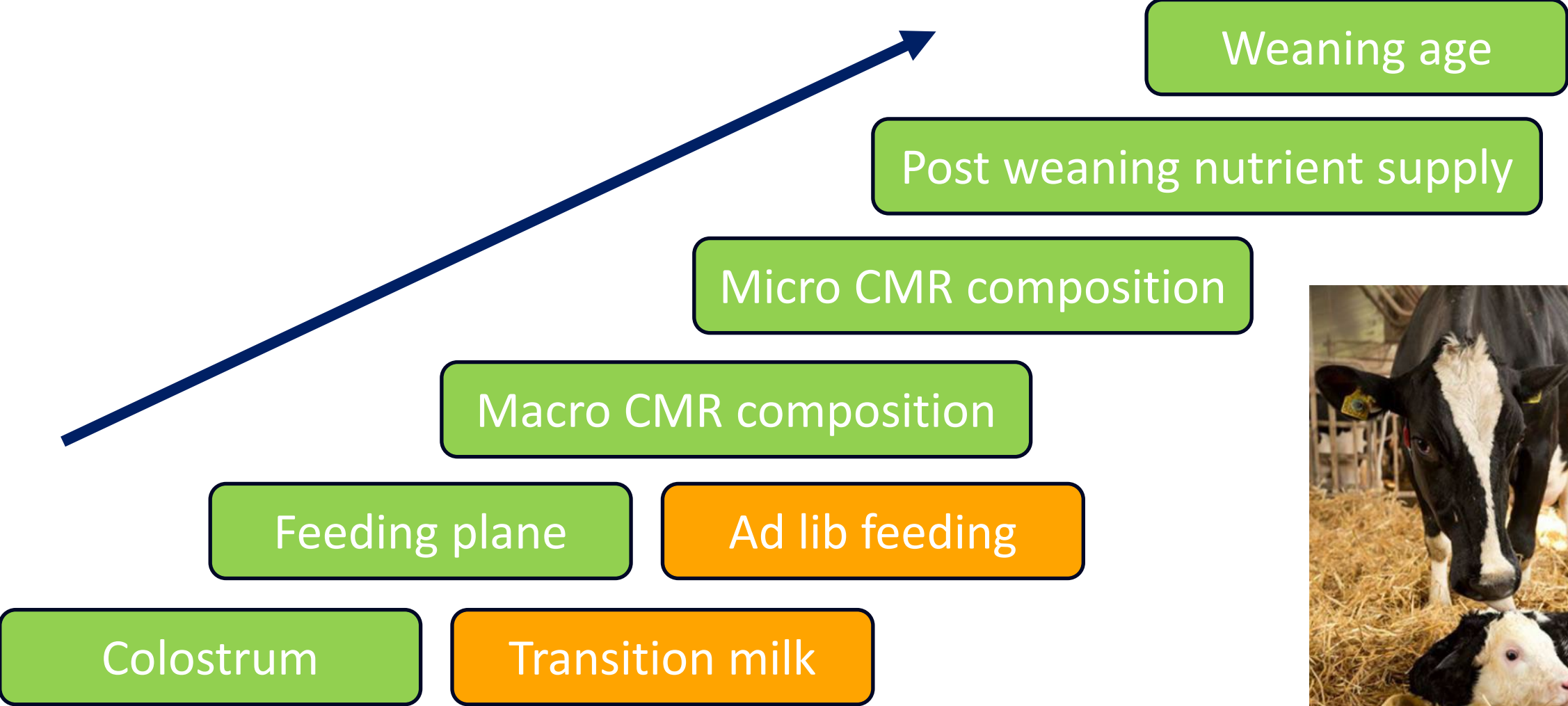
## Glucose tolerance



# Replacing milk is an exercise of nutrition and circularity



# LifeStart “restoration” ladder





# New value, new values, new objectives, new solutions

**LIFESTART**  
SETS LIFE PERFORMANCE

## OLD OBJECTIVES

Growth  
Feed conversion

Prewaning mortality

Solid feed intake  
Early weaning

Lean growth  
Rumen development

Fecal scores  
Antibiotic treatments

## NEW OBJECTIVES

Calf development  
and health

Metabolic resilience

GIT development  
Gut barrier function

Lifetime health

Lifetime productivity

## ADDITIONAL BENEFITS

Resource efficiency  
and circularity

Animal welfare

Reduced carbon  
intensity

Meeting societal  
expectations



# Take home messages

- “LifeStart” is a true case of metabolic programming
- Restoring perinatal environment restores phenotypical potential
- Milk does not only transfer nutrients, but also imprinting signals
- Improving perinatal environment, is the single greatest opportunity to improve dairy productivity, efficiency, health, and sustainability

OUR PURPOSE

*Feeding  
the Future*



OUR PURPOSE

*Feeding  
the Future*

**Thank you!**