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Changes in the plasma metabolome of Holstein heifers from birth to first insemination

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Introduction

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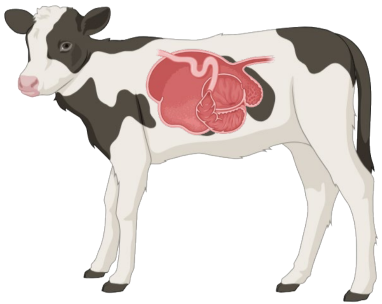
- Rearing period of calves is a key factor for their later productivity and lifespan
- Youngstock: additional requirements of energy for the development of the skeleton, muscles and organs & for an active immunity
- Calf growth and organ development stimulated by milk-feeding at levels close to ad libitum (Hammon et al., 2020)
- Wide ranging anatomical, physiological and microbial changes in the development of the gastrointestinal tract into a fully functional ruminant
    - Impact on digestion, absorption and intermediary metabolism (Meale et al., 2017)



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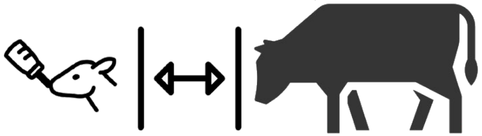
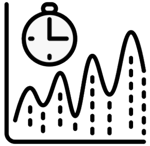
Introduction

- Metabolomics = identification and investigation of biochemical compounds for describing the metabolic activity of living tissue (Oliver et al., 1998)
- In dairy calves, no specific biomarkers have yet been identified that reflect the biological status at a certain point in time or predict a long-term effect (Leal et al., 2021)
- Associated changes in the metabolome are still poorly understood and are limited to the period up to weaning and shortly thereafter (Leal et al., 2021; Amin et al., 2023)
  - Effects of preweaning nutrient supply & age at weaning were shown to affect growth **and** the metabolome in blood

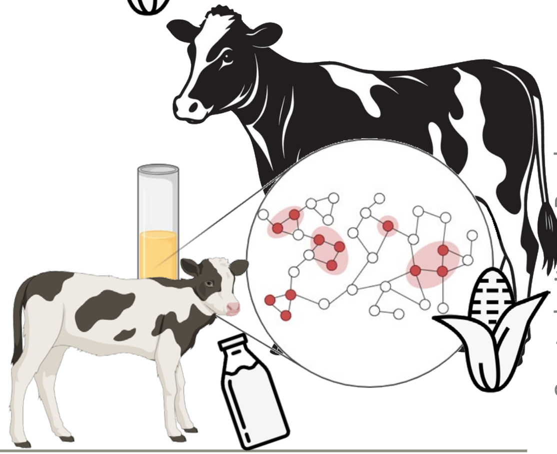
→ Research gap for the time after weaning 

Objectives

- Characterization of the plasma metabolome in Holstein dairy heifer calves 
- from birth throughout the rearing period until the first insemination 
- including the effects of the dietary changes related to weaning 

Hypothesis

- Substantial differences in the metabolite profile,
  especially when calves transition from liquid to solid feed



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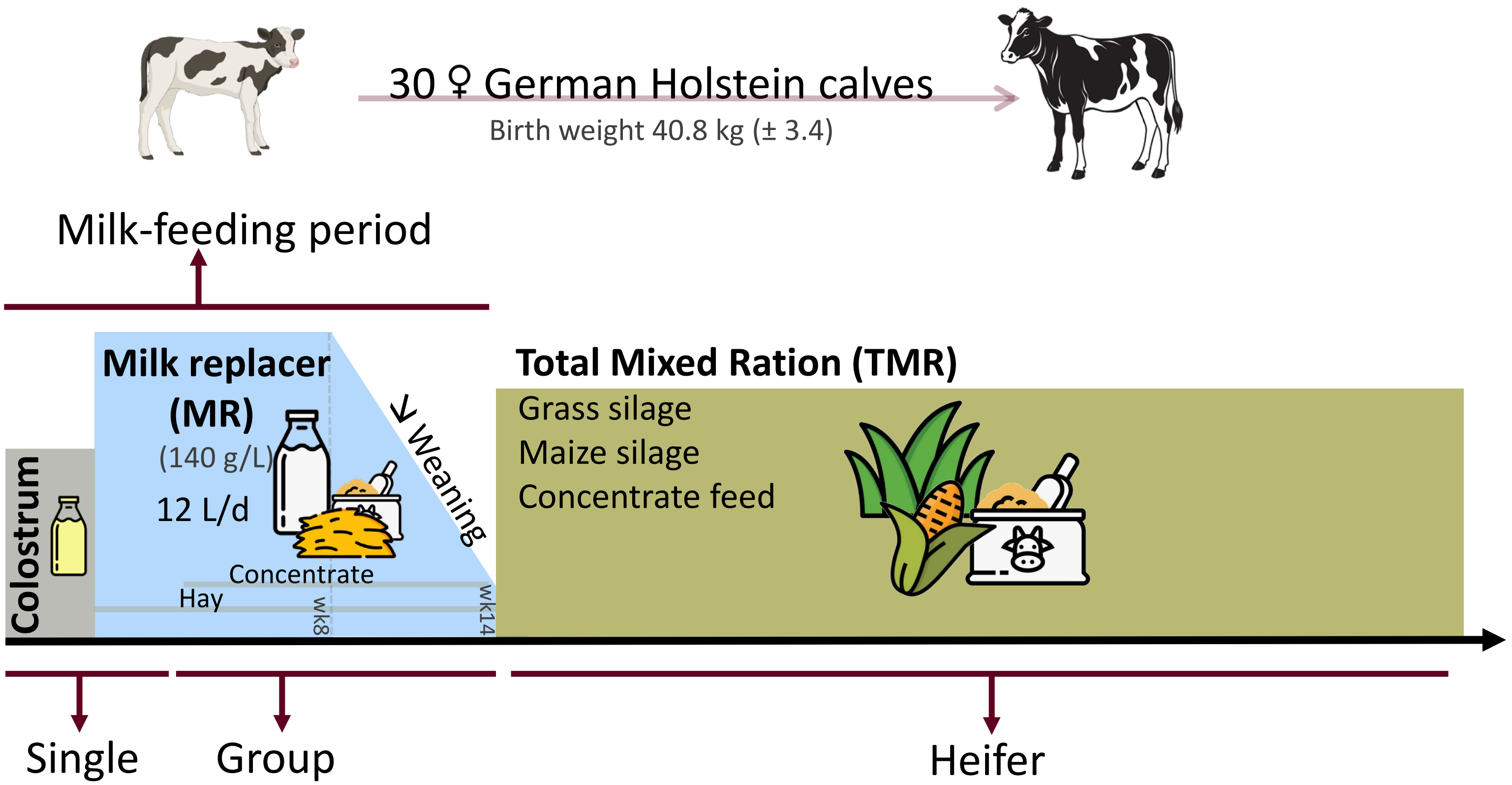
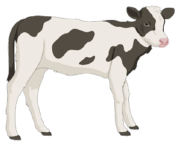
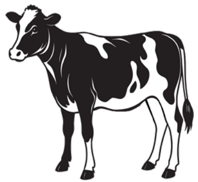
Animals and Feeding



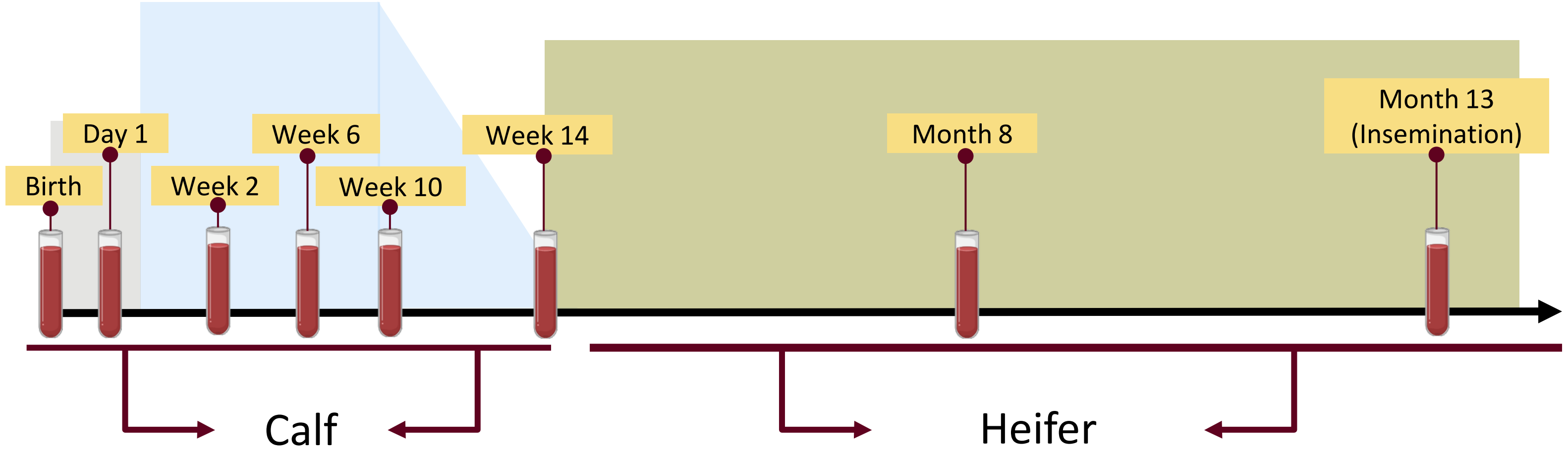
Blood sampling



(1) Birth
(2) Day 1
(3) Week 2
(4) Week 6
(5) Week 10
(6) Week 14



(7) Month 8
(8) Month 13 (Insemination)



Metabolomics

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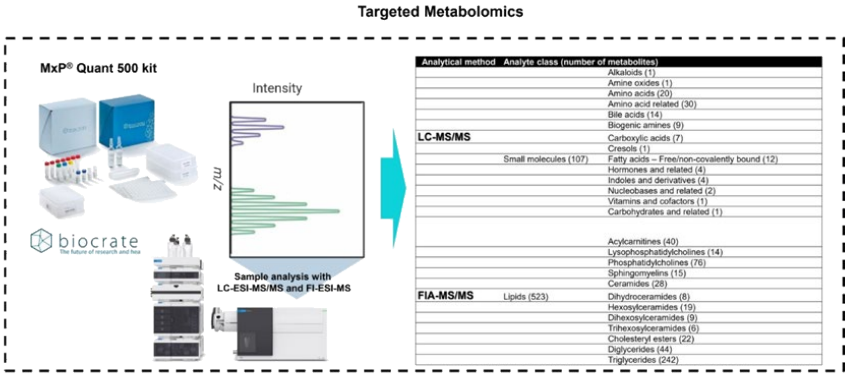
- Plasma obtained was immediately frozen and stored at -80°C
- MxP® Quant 500 kit (Biocrates Life Sciences AG, Innsbruck, Austria) was used to determine metabolite concentrations by tandem mass spectrometry
- Only metabolites with less than 20% missing values across all time points were used for analysis

  → **243 metabolites**

- summed with all related metabolites to obtain the sum per sample for each compound class



Ghaffari et al. (2024)

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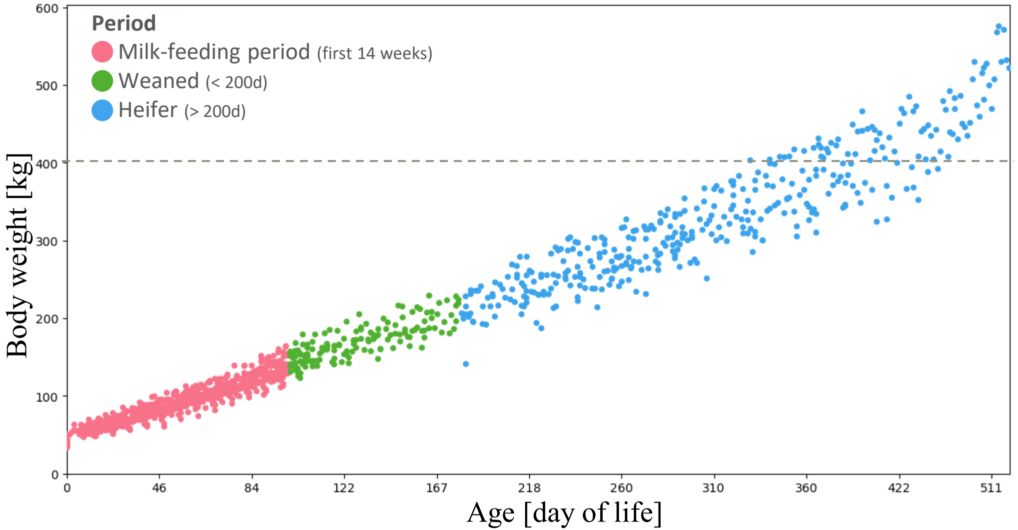
Body weight development

ADG milk-feeding period:	994 g/d
Weaning weight (14 weeks old):	135 ± 12.8 kg
Age 400 kg (insemination minimum):	395 ± 44 d



Metabolites in blood: Compound classes

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Glucose

Acylcarnitines

Biogenic Amines

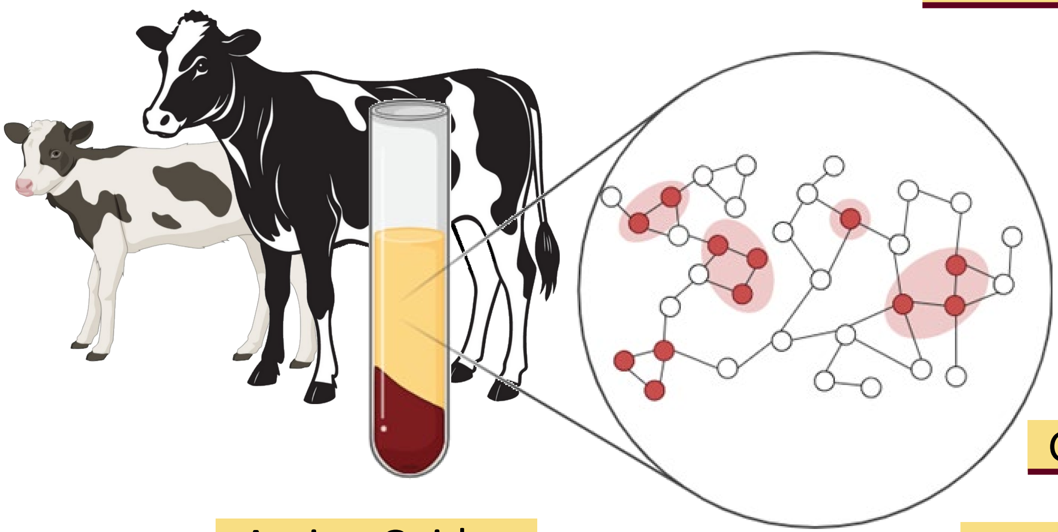
Bile Acids

Carboxylic Acids

Cresols

Amino Acids

Amino Acid Related



Sphingolipids

Cholesterol Esters

Glycerophospholipids

Amine Oxides

Vitamins

Triacylglycerols

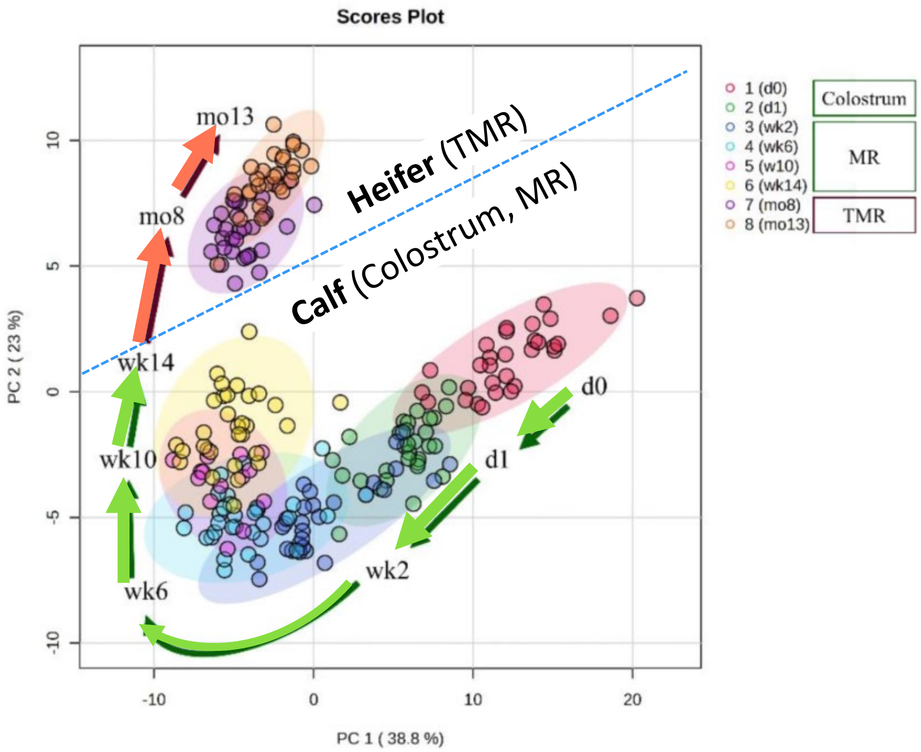
Ceramides

Glycosylceramides

Indoles and Derivatives

Metabolic profiles

- very distinct by time (aging)
- Most prevalent metabolite classes across all time points:
  - Glucose
  - Amino Acids (n=20)
  - Carboxylic Acids (n=2)
  - Cholesterol Esters (n=15)
  - Glycerophospholipids (n=73)



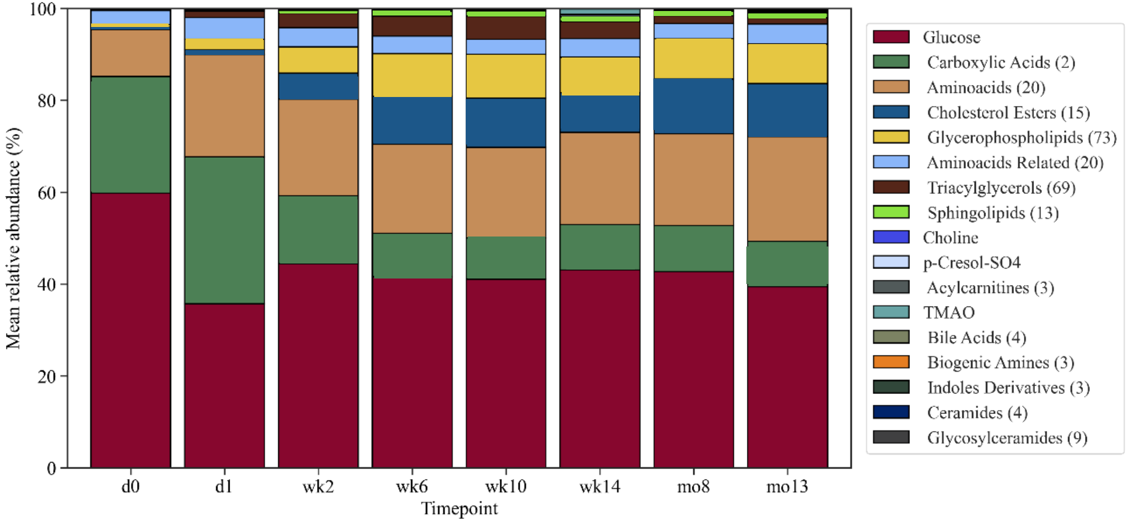
Principal component analysis (PCA) of metabolome data from birth (d0) to first insemination (mo13). Green arrows = calfhood, red arrows = heifer age

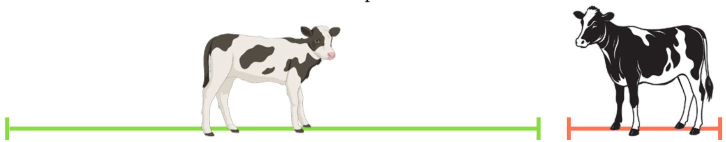
Prevalent metabolite classes

	1 (d0)	2 (d1)	3 (wk2)	4 (wk6)	5 (wk10)	6 (wk14)	7 (mo8)	8 (mo13)
1.	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose
2.	Carboxylic Acids	Carboxylic Acids	Amino Acids	Amino Acids	Amino Acids	Amino Acids	Amino Acids	Amino Acids
3.	Amino Acids	Amino Acids	Carboxylic Acids	Cholesterol Esters	Cholesterol Esters	Carboxylic Acids	Cholesterol Esters	Cholesterol Esters
4.	Amino Acids Related	Amino Acids Related	Glycero-phospholipids	Carboxylic Acids	Glycero-phospholipids	Cholesterol Esters	Carboxylic Acids	Carboxylic Acids
5.	Glycero-phospholipids	Glycero-phospholipids	Cholesterol Esters	Glycero-phospholipids	Carboxylic Acids	Glycero-phospholipids	Glycero-phospholipids	Glycero-phospholipids

Relative abundance of compound classes

- The development of the heifers was associated with
  - Carboxylic Acids
  - Cholesterol Esters
  - Glycerophospholipids
  - Sphingolipids





Changes of each class

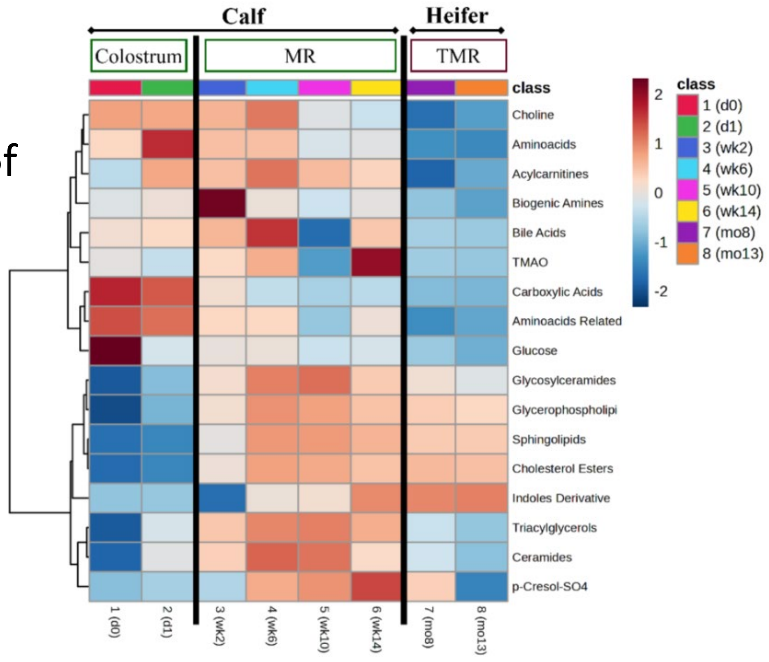
- An age effect was identified in each class of compounds analyzed

Calf vs. Heifer

- Preweaned calves higher total concentrations of
  - Amino Acids
  - Amino Acid-related metabolites
  - Acylcarnitines
  - Choline

Weaning

- p-Cresol-SO4 (Week 14)
- Indoles and Derivatives (Week 14)



Heatmap analysis visualizing the metabolic changes of the compound classes in Holstein dairy heifers due to heifers' age.

Peaks in concentration during milk-feeding period

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At one or two consecutive time points:

- Biogenic Amines Week 2
- Bile Acids Week 6
- Ceramides
  Week 6 & Week 10
- Trimethylamine N-oxide (TMAO) Week 14

Class	1 (d0)	2 (d1)	3 (wk2)	4 (wk6)	5 (wk10)	6 (wk14)	7 (mo8)	8 (mo13)
				Timepoint				
Glucose	18253	6212^{ac}	6760^{c}	6845^{ac}	5905^{abc}	6200^{a}	5013^{a}	4409^{b}
Carboxylic Acids	8225^{a}	5606^{b}	2269^{c}	1582^{c}	1295^{c}	1441^{c}	1207^{c}	1074^{c}
Amino Acids	3159^{a}	3884^{b}	3182^{a}	3194^{a}	2839^{ae}	2884^{ac}	2331^{cd}	2230de
Cholesterol Esters	137^{a}	185^{a}	866	1750^{b}	1537^{bc}	1254^{c}	1399^{c}	1248^{c}
Glycerophospholipids	172	393	879^{a}	1541^{b}	1378^{b}	1117^{c}	1033^{ac}	912^{ac}
Amino Acids Related	884^{a}	820^{a}	635^{b}	630^{b}	461^{cd}	589^{bc}	387de	406^{e}
Triacylglycerols	57^{a}	246^{a}	492^{c}	739^{d}	727^{d}	542^{cd}	195^{a}	124^{a}
Sphingolipids	31^{a}	36^{a}	90	162^{b}	159^{b}	141^{bc}	122^{cd}	115^{d}
Choline	21.01^{a}	20.47^{abd}	20.67^{acd}	23.32^{bc}	15.07^{de}	14.50^{e}	8.84^{f}	10.90^{ef}
P-Cresol-SO4	2.92^{a}	3.93^{a}	6.03	18.32	31.97 ^{b}	47.76^{c}	42.89^{bcd}	49.72^{d}
Acylcarnitines	9.87a	18^{bcde}	16^{bdf}	22^{c}	16^{dg}	14^{efg}	6.54^{a}	8.8^{a}
TMAO	5.08^{a}	2.69^{a}	11.16^{a}	17.09^{a}	11.81^{a}	169.85	6.83^{a}	7.10^{a}
Bile Acids	3.4^{a}	3.61^{a}	5.81^{a}	16.4	0.81^{a}	5.64^{a}	1.44^{a}	1.43^{a}
Biogenic Amines	3.31^{a}	3.59^{a}	7.36	3.61^{a}	3.1^{a}	3.39^{a}	2.69^{a}	2.51^{a}
Indoles Derivatives	1.21^{a}	1.34^{abcd}	0.74^{ab}	2.99^{cd}	3.37^{d}	6.83^{e}	6.75^{e}	6.9^{e}
Ceramides	0.25^{a}	0.67^{bc}	0.84^{b}	1.42^{d}	1.31^{d}	0.78^{bc}	0.58^{ce}	0.42^{ae}
Glycosylceramides	0.88	1.50	2.56^{a}	4.18^{b}	4.40 ^{b}	3.01^{ac}	2.52^{acd}	2.16^{ad}

$^{a–f}$ Means (µmol/L) within a row with different letters differ ($P < 0.001$).

Affected biological processes

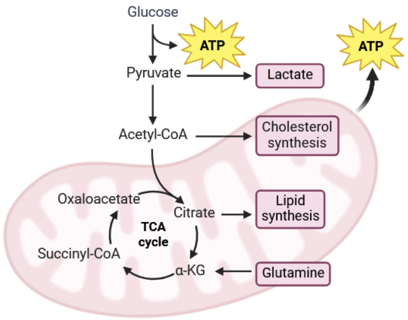
Protein metabolism

- Amino Acids
- Amino Acid related metabolites

Lipid metabolism

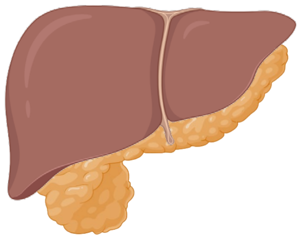
- Cholesterol Esters
- Glycerophospholipids
- Acylcarnitines
- Sphingolipids
- Carboxylic acids
- (Choline)

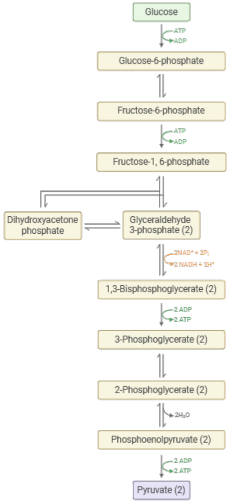
Energy metabolism



Carbohydrate metabolism

- Glucose





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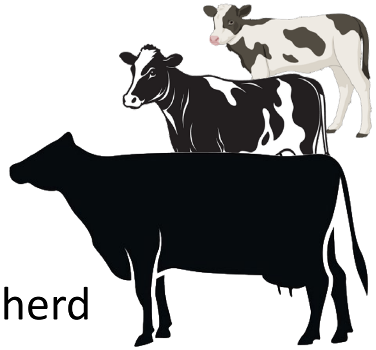
Conclusion

- Plasma metabolome of Heifers undergoes ongoing changes during growth
  → Availability of nutrients and physical development during this period are closely linked to later production performance
- First study characterizing these changes during the first year of life using detailed metabolome profiling at 8 time points
- Gap of knowledge between weaning and first calving
  → at least partly filled with our analyses from the 8th to the 13th month of life

Outlook

- The same heifers were further monitored until first calving
  → additional insights into the relation between the rearing period and later development and performance upon entering the dairy herd



Thank you for your attention!

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