Serum lipidome profiling of dairy calves fed high-fat milk replacers containing animal or vegetable fats

M. H. Ghaffari [1], J. N. Wilms [2], D. Caruso [3], H. Sauerwein [1], L. N. Leal [2]

[1] *Institute of Animal Science, University of Bonn, Germany.*
[2] *Trouw Nutrition Research and Development, Amersfoort, the Netherlands*
[3] *Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano Via Balzaretti, Milano, Italy*

EAAP European Federation of Animal Science

75th Annual Meeting of the European Association for Animal Production (EAAP)
Florence, Italy | September 1st - 5th, 2024

trouw nutrition a Nutreco company

UNIVERSITÄT BONN

landwirtschaftliche fakultät

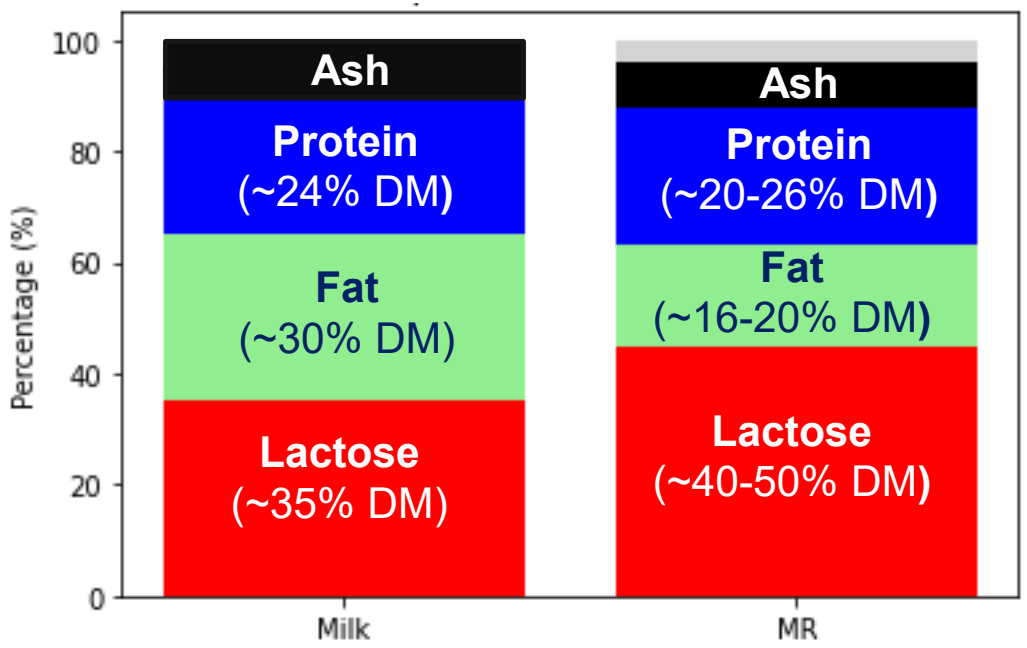
Background

Milk or milk replacers (MR)

- Primary nutrient source for growth of neonatal calves

Macronutrient composition of MR vs. whole milk

- Higher lactose
- Lower fat
- Variable protein (same, lower, or higher)



Daniels et al., 2008; USDA, 2011 ; Echeverry-Munera et al., 2021; Wilms et al., 2022

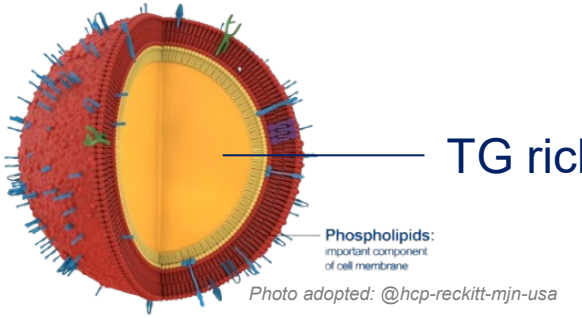
Photo by Ghaffari, 2024

Background

Milk fat: best for calves

- ~98% triglycerides (TG)
- ~ 400 fatty acids (FA)

Jensen et al.,1990

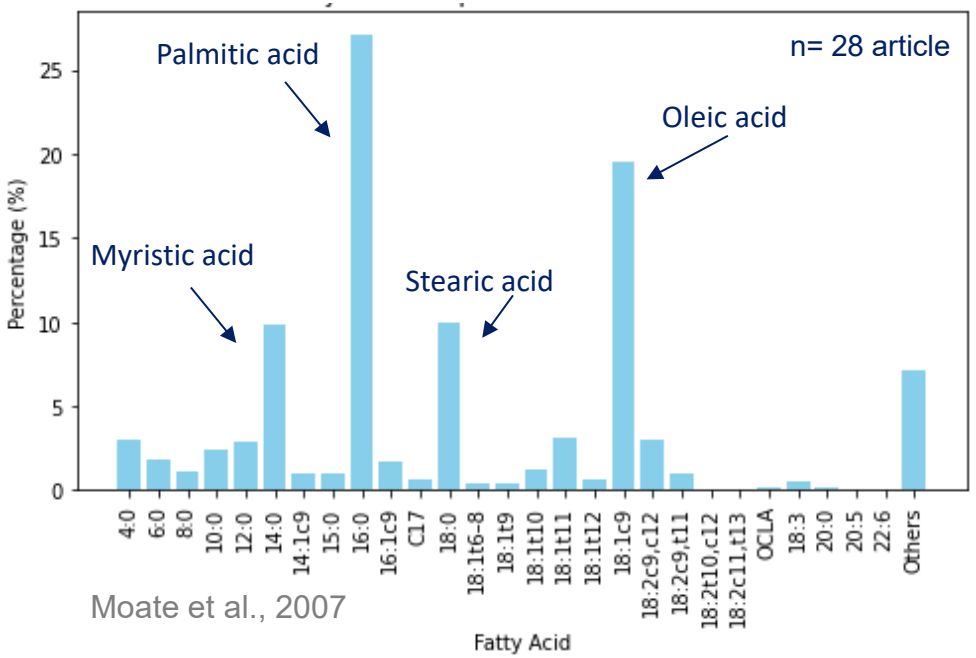


Milk fat composition:

- Saturated FA: ~ 67%
- Monounsaturated FA: ~ 25%
- Polyunsaturated FA: ~ 2-3%
- Short and Medium-chain FA: ~10-15% (highly digestible)

Hageman et al., 2019

Whole milk



Moate et al., 2007

Milk replacer fat:

- FA profiles and TG structures vary by fat source

Background

Fat sources in calf milk replacer

- **Europe & other regions:** Primarily vegetable fats

- **North America:** Fat blends with lard, tallow, and coconut

Huuskonen et al., 2005 ; Drackley, 2008; Wilms et al., 2022 ; Mellors et al., 2023

Palm oil

Coconut oil

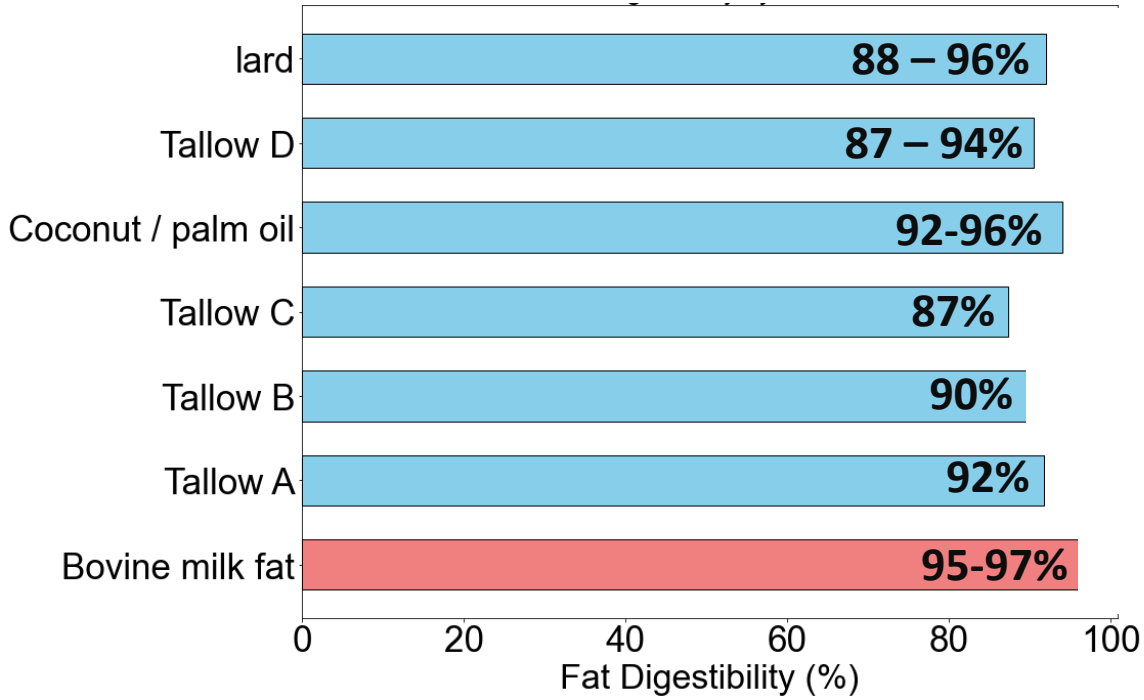
Rapeseed oil

Tallow

Lard

Digestibility of various fat sources

- Highly digestible in MR with proper formulation



Raven, 1970; Toullec et al., 1980; Lalle´s et al,1995; Guilloteau et al, 1986; Wood, 2022

Knowledge gap:

- Insufficient studies on high-fat milk replacers (MR)
- Limited understanding of how fat sources in MR affect serum lipids in calves

Study objective:

- Analyze serum lipids in calves fed high-fat (30%) MR with vegetable or animal fats
- Use LC-QTOF-MS for lipidomic analysis

LC-QTOF-MS : liquid chromatography-quadrupole time-of-flight mass spectrometry

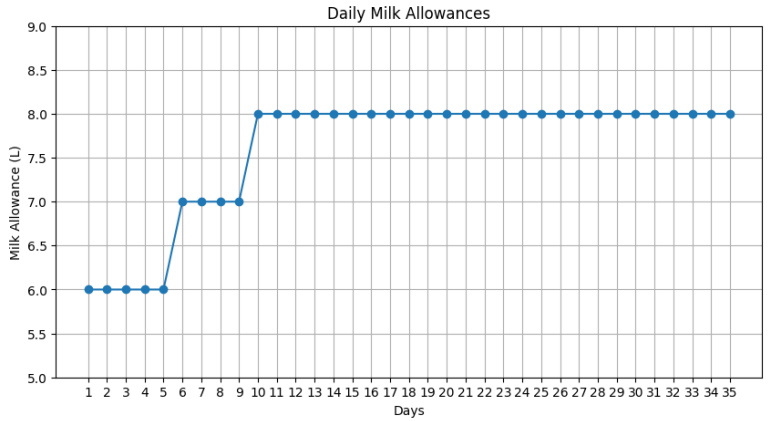
Animals and Experimental Design

- Conducted at Trouw Nutrition Research and Development in 2020 (the Netherlands)
- 30 Holstein-Friesian male calves (0-3 days old) were purchased in batches from 7 nearby dairy farms.
- Colostrum (Brix value of 22%) 3.0-4.0 L within 3 h, 2 feedings of 2 L (in 24 h)

- Calves assigned to 15 blocks based on arrival day and age
- Randomly assigned to 2 experimental diets within each block (n = 15/group)

Milk allowance (twice daily at 135 g/L):

- **6.0 L/day**: days 1-5 after arrival
- **7.0 L/day**: days 6-9
- **8.0 L/day**: days 10-35
- No starter feed; ad libitum water and chopped wheat straw



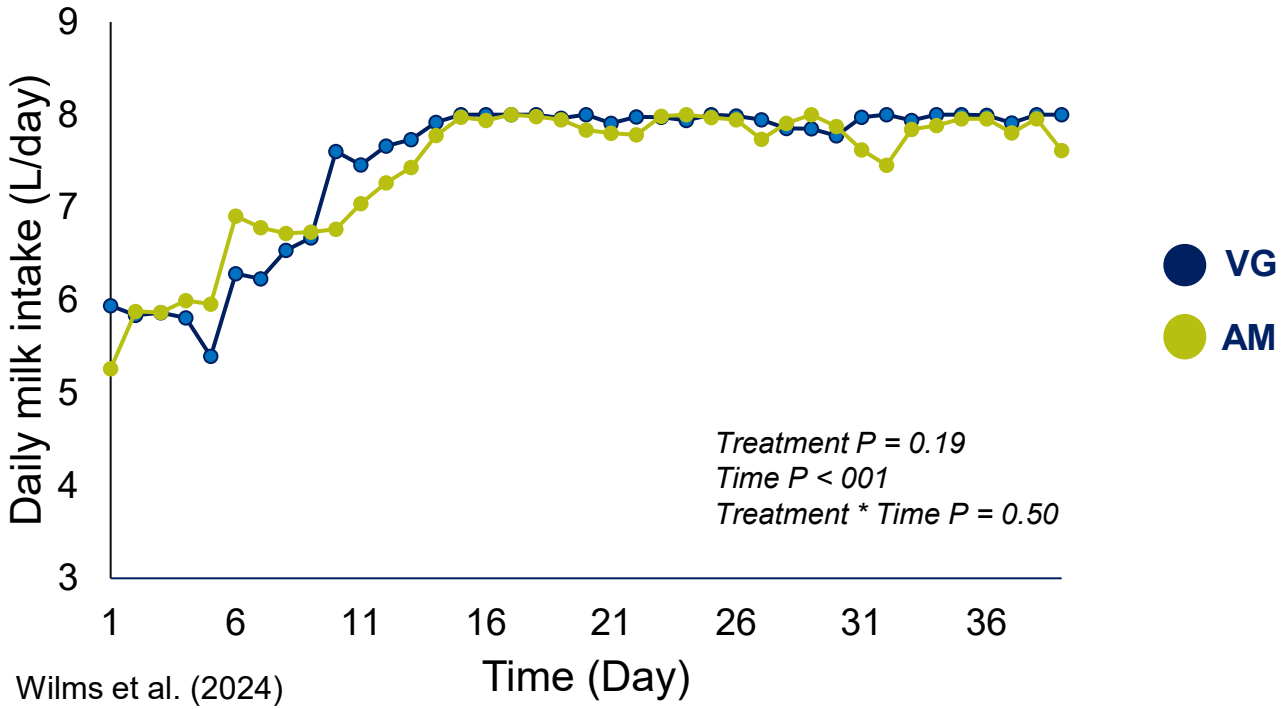
Wilms et al. (2024)

Experimental design

Treatment diets: milk replacer (MR) with different fat sources

- Vegetable fats: **VG**; 65% rapeseed oil, 35% coconut oil
- Animal fats: **AN**; 65% lard, 35% dairy cream)

- Both MR were isoenergetic (~ 22.3 MJ/kg), with 30% fat, 24% crude protein, and 36% lactose as DM

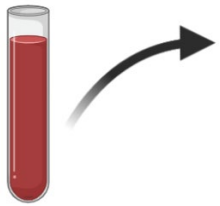


Wilms et al. (2024)

Overview of the sampling and lipidomic analysis

A

Blood sampling
(week 4 of age)

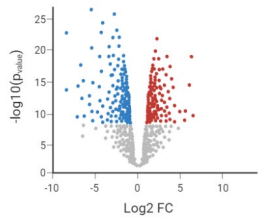


VG

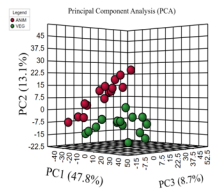
AN

(15 calves/treatment)

B



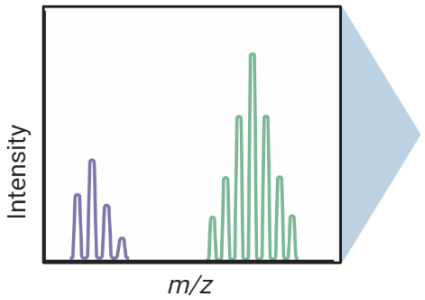
Volcano plot graph



Lipids classified in 25 classes

Phosphatidylcholine
Sphingomyelin
Phosphatidylethanolamine
Triacylglycerol
Fatty acid + Fatty acid ester of hydroxyl fatty acid
Phosphatidylinositol
Lysophophatidylcholine
Lysophosphatidylethanolamine + N-acyl lysophosphatidylethanolamine
Diacylglycerol
N-acyl ethanolamines
Hexosylceramide
Sterol
Ceramide phosphoethanolamine
Phosphatidylglycerol
Ganglioside GM3
Ceramide
Sulfatide
Dihexosylceramide
Lysophosphatidylinositol
Monogalactosyldiacylglycerol
Coenzyme Q
Hydroxyeicosatetraenoic acids
Lysophosphatidic acid

Lipidomic analysis

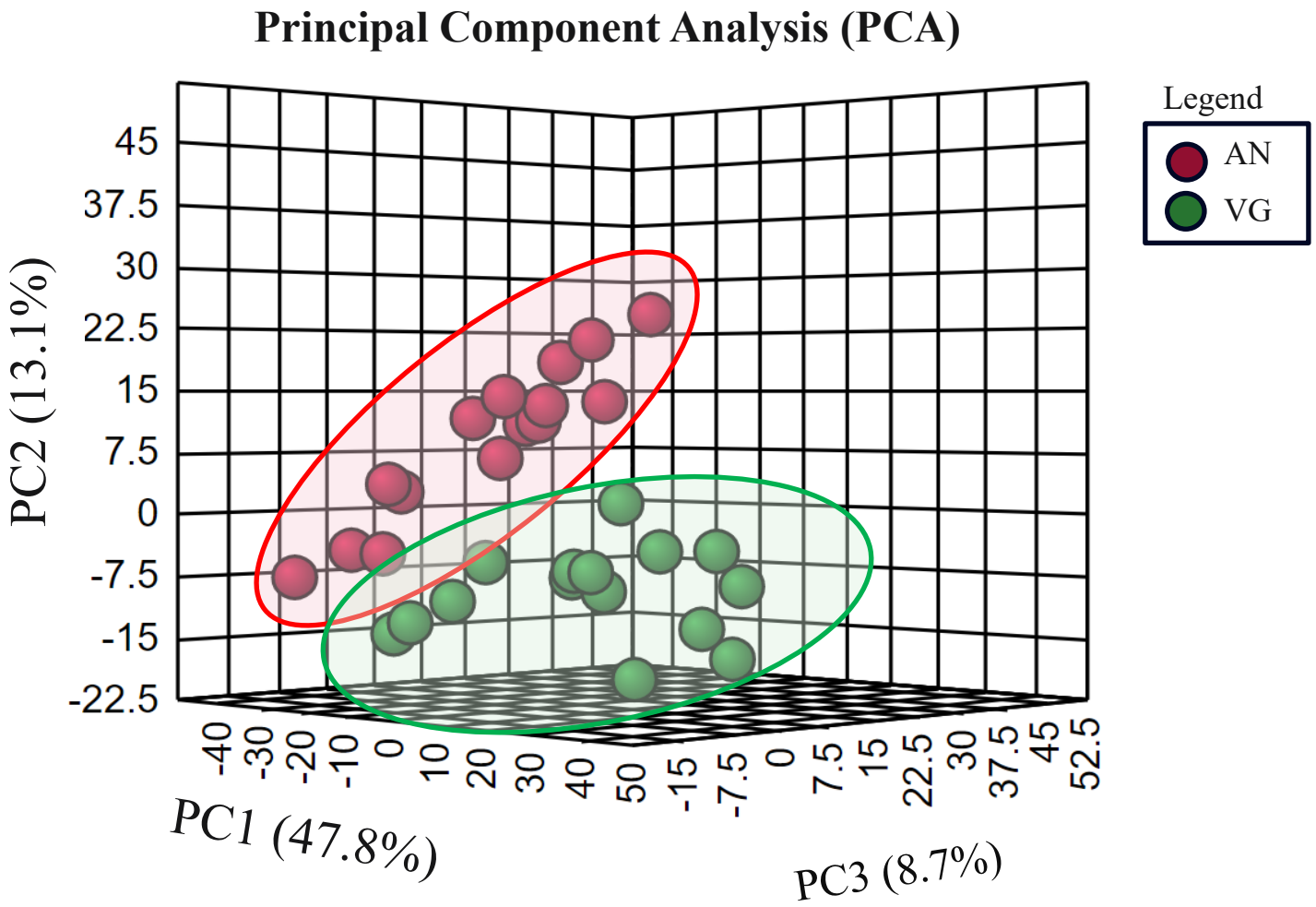


Liquid Chromatography (LC) connected to an ExionLC™ AD system (ABSciex) and analyzed in a TripleTOF 6600 mass spectrometer (Quadrupole Time-Of-Flight, QTOF - ABSciex)

Ghaffari et al. 2024 JDS

Results

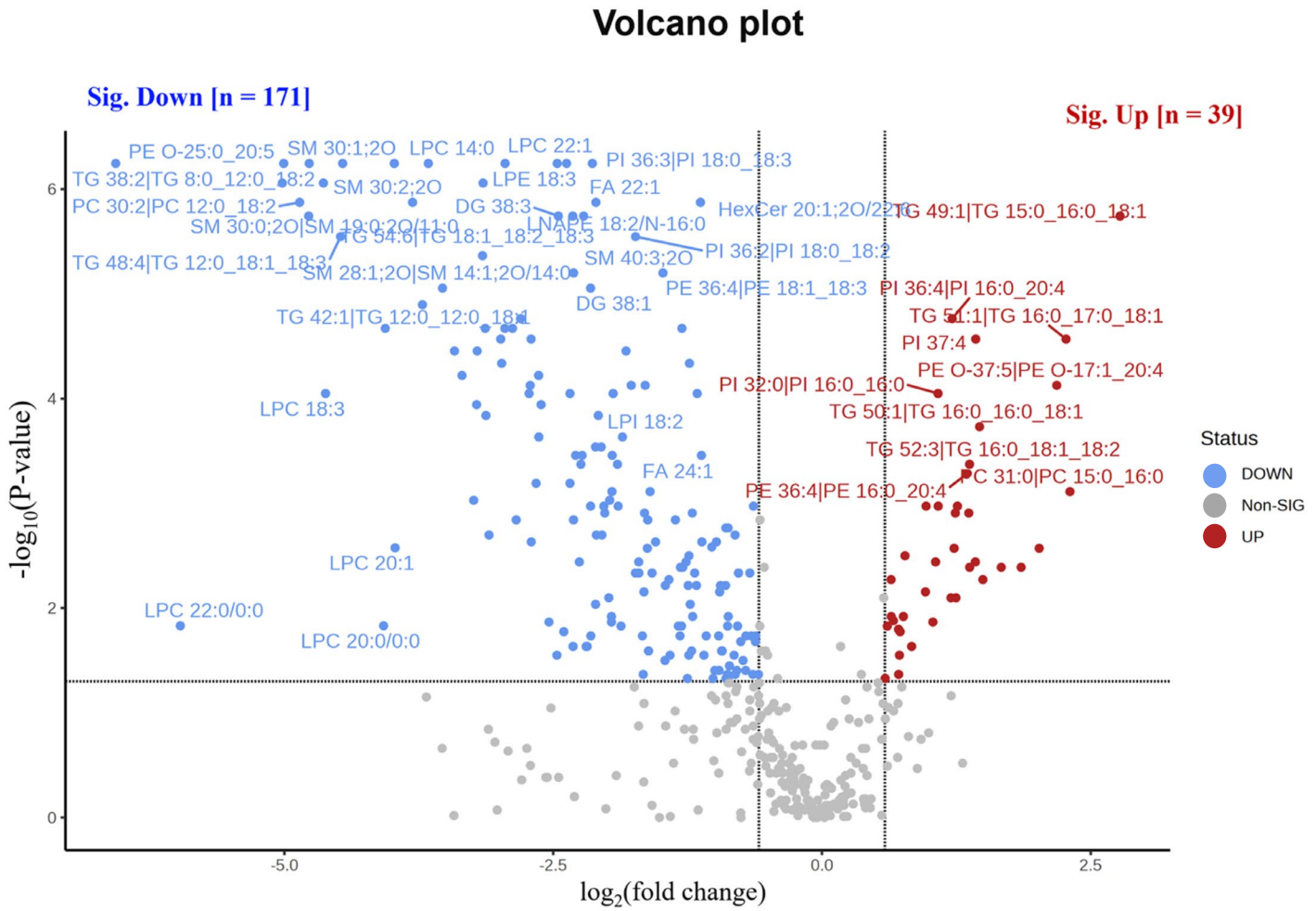
- A total of 594 lipids across 25 classes



Ghaffari et al. 2024 JDS

Results

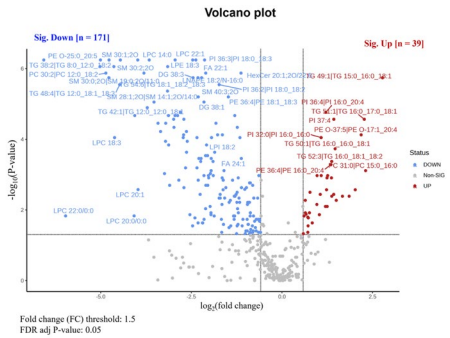
- 210 lipid species significantly affected by fat source in milk replacers



Fold change (FC) threshold: 1.5
FDR adj P-value: 0.05
Comparison: AN vs VG

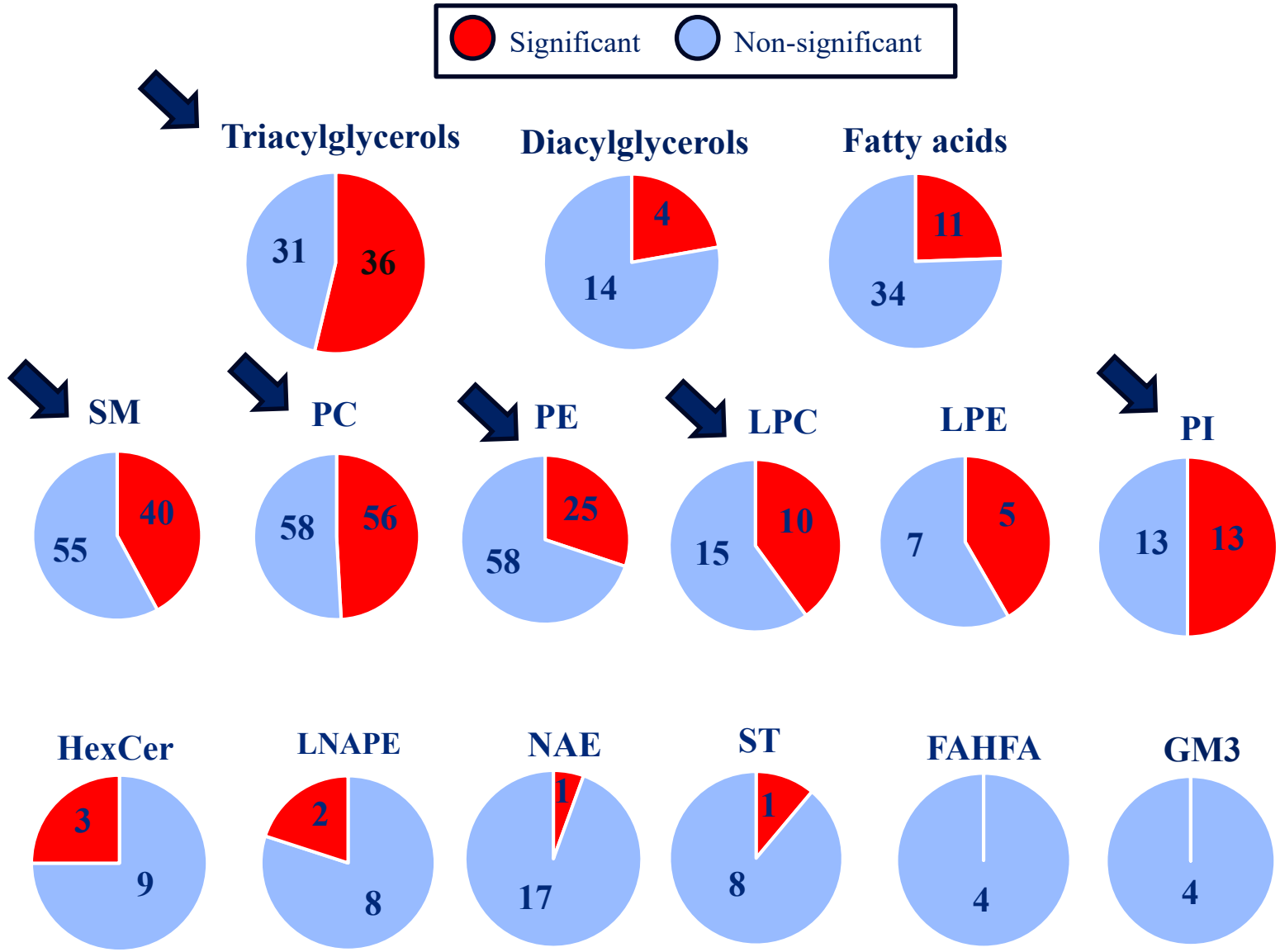
Ghaffari et al. 2024 JDS

Results



Sphingomyelins (**SM**)
Phosphatidylcholines (**PC**)
Phosphatidylethanolamines (**PE**)
Lysophosphatidylcholines (**LPC**)
Lysophosphatidylethanolamines (**LPE**)
Phosphatidylinositols (**PI**)

Hexosylceramides (**HexCer**)
n-acyl-LPE (**LNAPE**)
n-acyl ethanolamines (**NAE**)
Sterols (**ST**),
Fatty acid esters of hydroxy fatty acids (**FAHFA**)
Ganglioside (**GM3**)



The pie charts show significant lipid species by class for calves fed AN vs. VG.

Results

Fatty acids (FA) profile in AN vs VG milk replacer

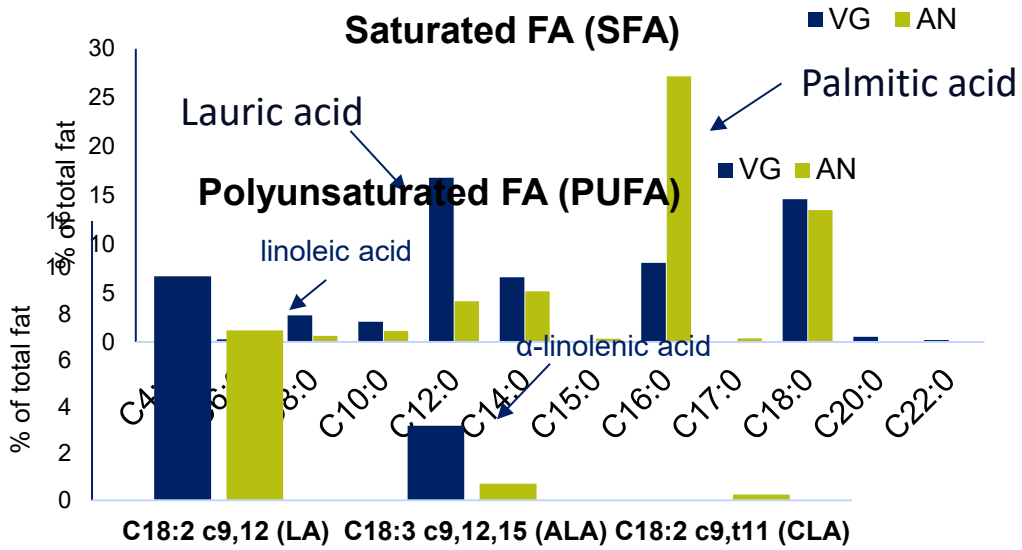
Same level of **total Saturated FA** (54% vs. 52%)

Lower medium-chain SFA in AN vs. VG (C8, C10, C12)

Higher long-chain SFA in AN vs. VG (mainly C16)

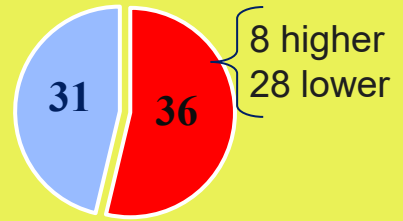
Same level of **total Monounsaturated FA** (34.9% vs. 34.2%)

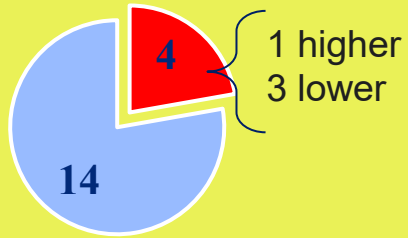
Lower level of **Polyunsaturated FA**

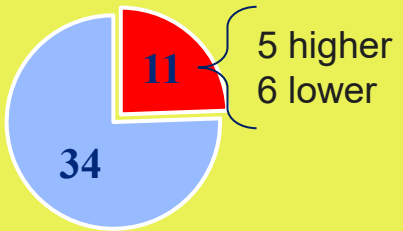


Serum lipids

AN vs VG

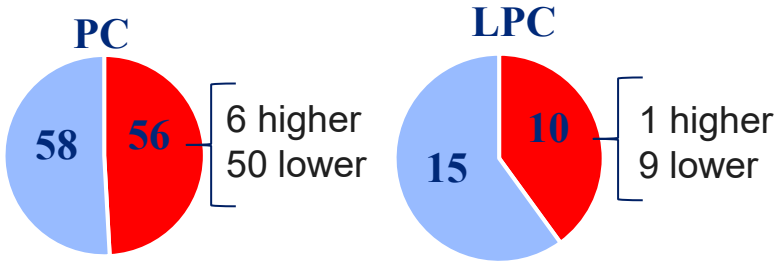






Ghaffari et al. 2024 JDS

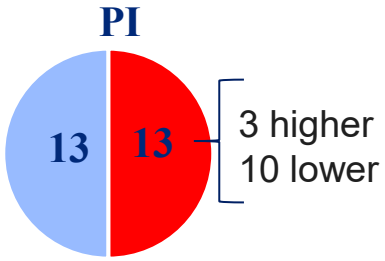
Results



Phosphatidylcholines (PC)

- Role: cell membrane integrity, function, and signaling

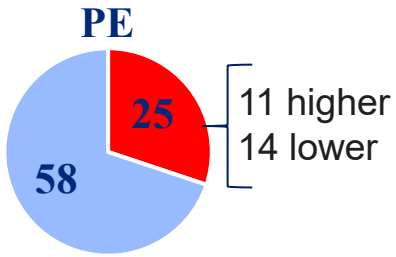
Morita et al., (2010); Vance, (2002)



Phosphatidylinositol (PI)

- Role: Signal transduction and intracellular transport

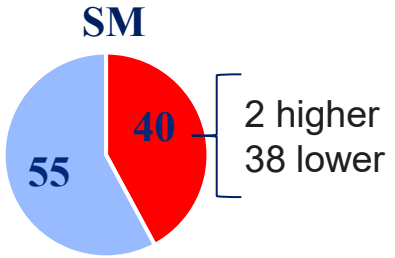
Di Paolo and De Camilli, (2006); Blunsom and Cockcroft, (2020)



Phosphatidylethanolamines (PE)

- Role: cell division and membrane dynamics

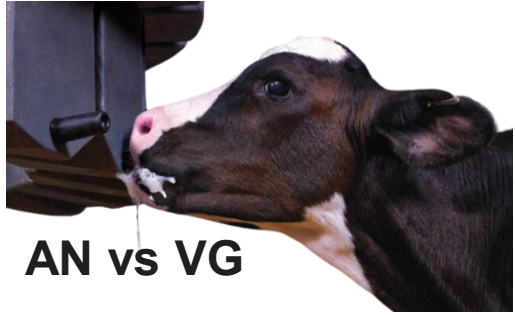
Farine et al., (2015)



Sphingomyelins (SM)

- Synthesized in liver's endoplasmic reticulum
- Role: structural integrity and function of lipoproteins

Deevska et al., (2012); Iqbal et al., (2017)



alter the structural and functional properties of lipoproteins, potentially affecting lipid transport and metabolism

Take home massage

- **Influence of fat source in milk replacers:** significant influence on serum lipid profiles

- **Lipid changes:** Animal-fed calves had lower levels of key lipid species (PC, PE, PI, SM), indicating shifts in lipoprotein and lipid metabolic pathways

- **Uncertainty:** Unclear if these alterations benefit or harm calf development, highlighting need for further research

Ghaffari et al. 2024 JDS

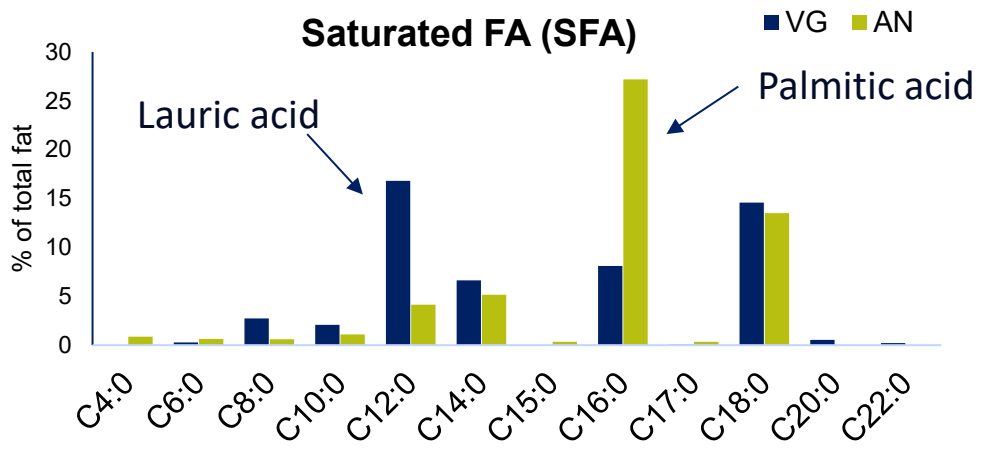
J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2024-25120

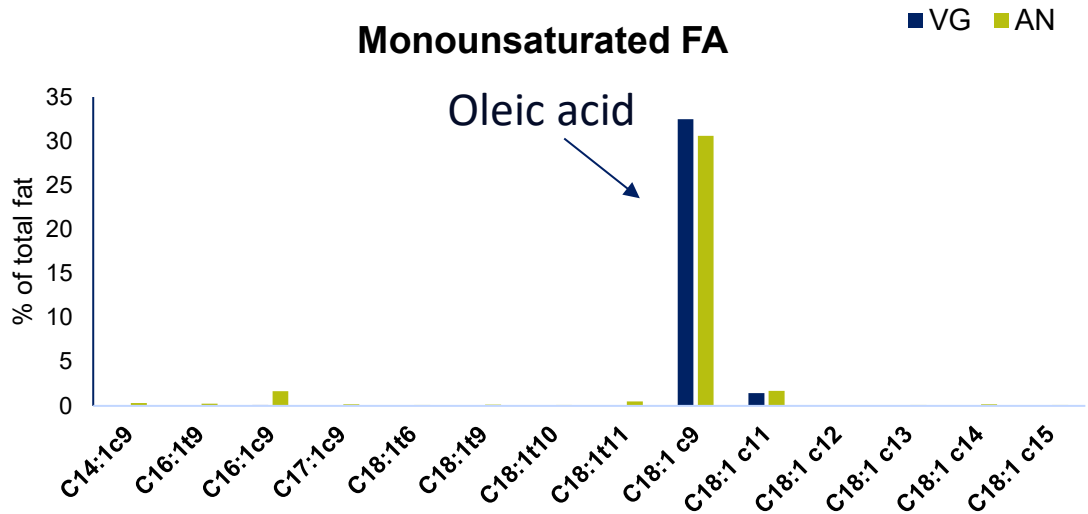
Serum lipidomic profiling of dairy calves fed milk replacers containing animal or vegetable fats

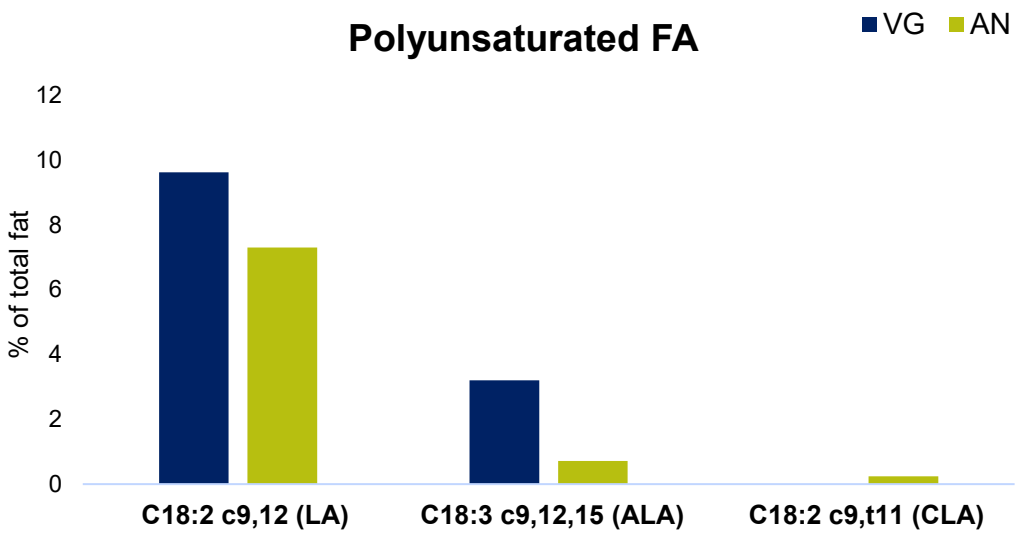
M. H. Ghaffari,[1]*# J. N. Wilms,[2]# D. Caruso,[3,4] H. Sauerwein,[1] and L. N. Leal[2]*
[1]Institute of Animal Science, University of Bonn, 53111 Bonn, Germany.
[2]Trouw Nutrition Research and Development, P.O. Box 299, 3800 AG, Amersfoort, the Netherlands.
[3]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano Via Balzaretti, 20133 Milano, Italy.
[4]Unitech OMICs, Mass Spectrometry Platform, University of Milan, Milan, Italy.

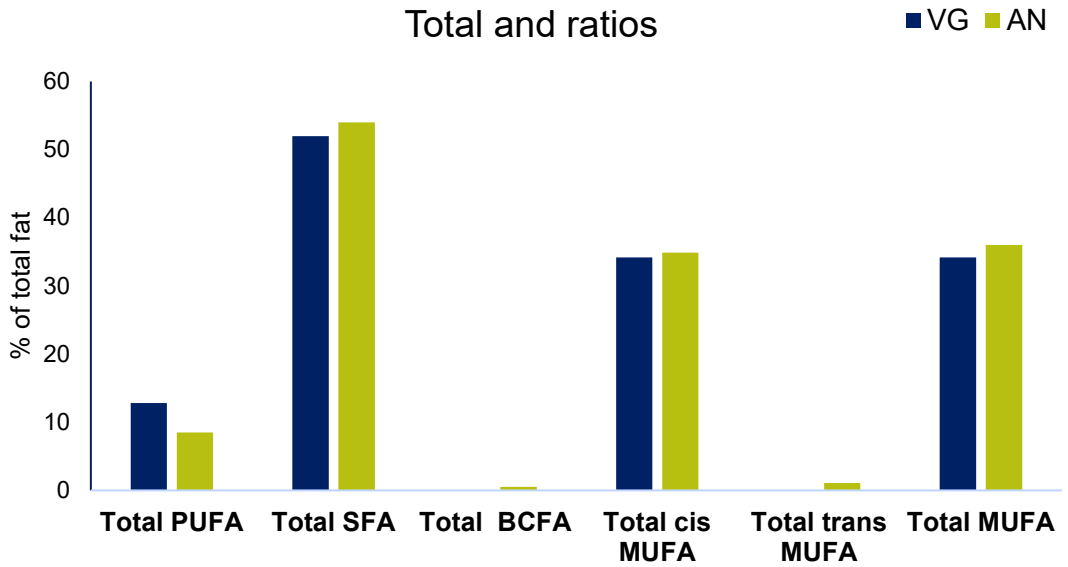
Email: morteza1@uni-bonn.de

Fatty acid profiles of milk replacers



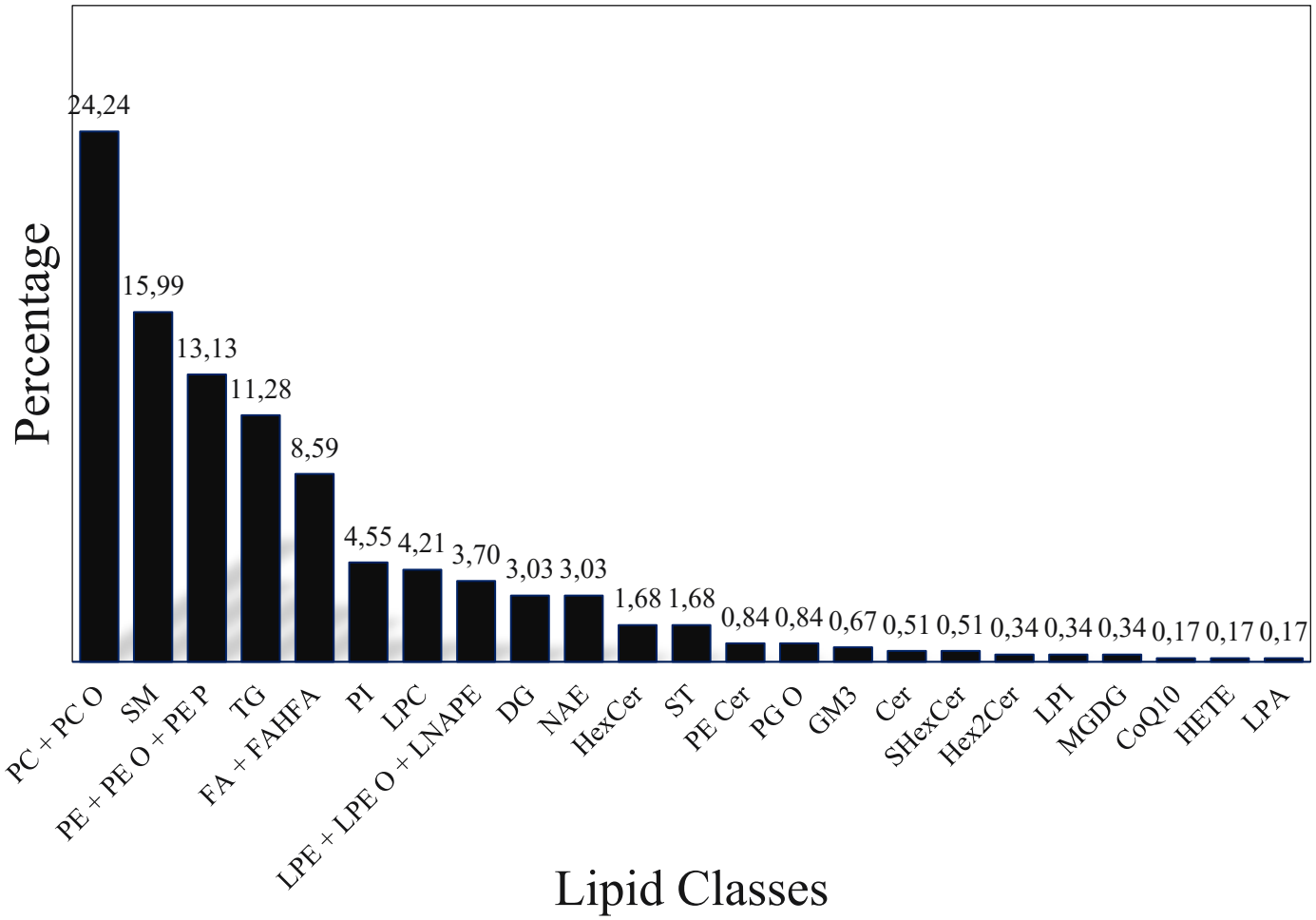






Wilms et al. (2024)

Distribution of lipid classes in calf serum lipidome profiles analyzed by LC-QTOF-MS.



Ghaffari et al. 2024 JDS

Phosphatidylcholine	PC + PC O
Sphingomyelin	SM
Phosphatidylethanolamine	PE + PE O + PE P
Triacylglycerol	TG
Fatty acid + Fatty acid ester of hydroxyl fatty acid	FA + FAHFA
Phosphatidylinositol	PI
Lysophophatidylcholine	LPC
Lysophosphatidylethanolamine + N-acyl lysophosphatidylethanolamine	LPE + LPE O + LNAPE
Diacylglycerol	DG
N-acyl ethanolamines	NAE
Hexosylceramide	HexCer
Sterol	ST
Ceramide phosphoethanolamine	PE Cer
Phosphatidylglycerol	PG O
Ganglioside GM3	GM3
Ceramide	Cer
Sulfatide	SHexCer
Dihexosylceramide	Hex2Cer
Lysophosphatidylinositol	LPI
Monogalactosyldiacylglycerol	MGDG
Coenzyme Q	CoQ10
Hydroxyeicosatetraenoic acids	HETE
Lysophosphatidic acid	LPA