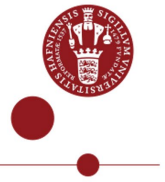
Sunde og produktive nykælvere WP3

Project and status meeting

Aarhus, 23/11/2023

Postdoc: Paraskevi Tsermoula
Associate Prof.: Bekzod Khakimov



KØBENHAVNS UNIVERSITET

Colostrum NMR metabolomics

Aim

- To characterize the molecular composition of bovine colostrum.
- To identify markers that reflect the physiological status of the cows around calving.

Samples (259 samples)

- 2021
- 2022
- No analysis for 2023 due to low number of samples

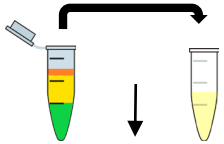
Sample preparation

Thaw at 40°C for 30 min

Dilute 1:4, v/v

Centrifuge
(1.5K g, 30', 4°C)

400 µl of middle layer in new tube



Centrifuge with 10kDa cut-off filter
(21K g, 30', 4°C)

400 µl serum + 200 µl D_20
(8 mg/ml TSP, 0.13 mg/ml NaN_3)

Tranfer 550 µl
in NMR tube

Milk serum

Measure

Ultrafiltered colostrum ^{1}H NMR spectrum

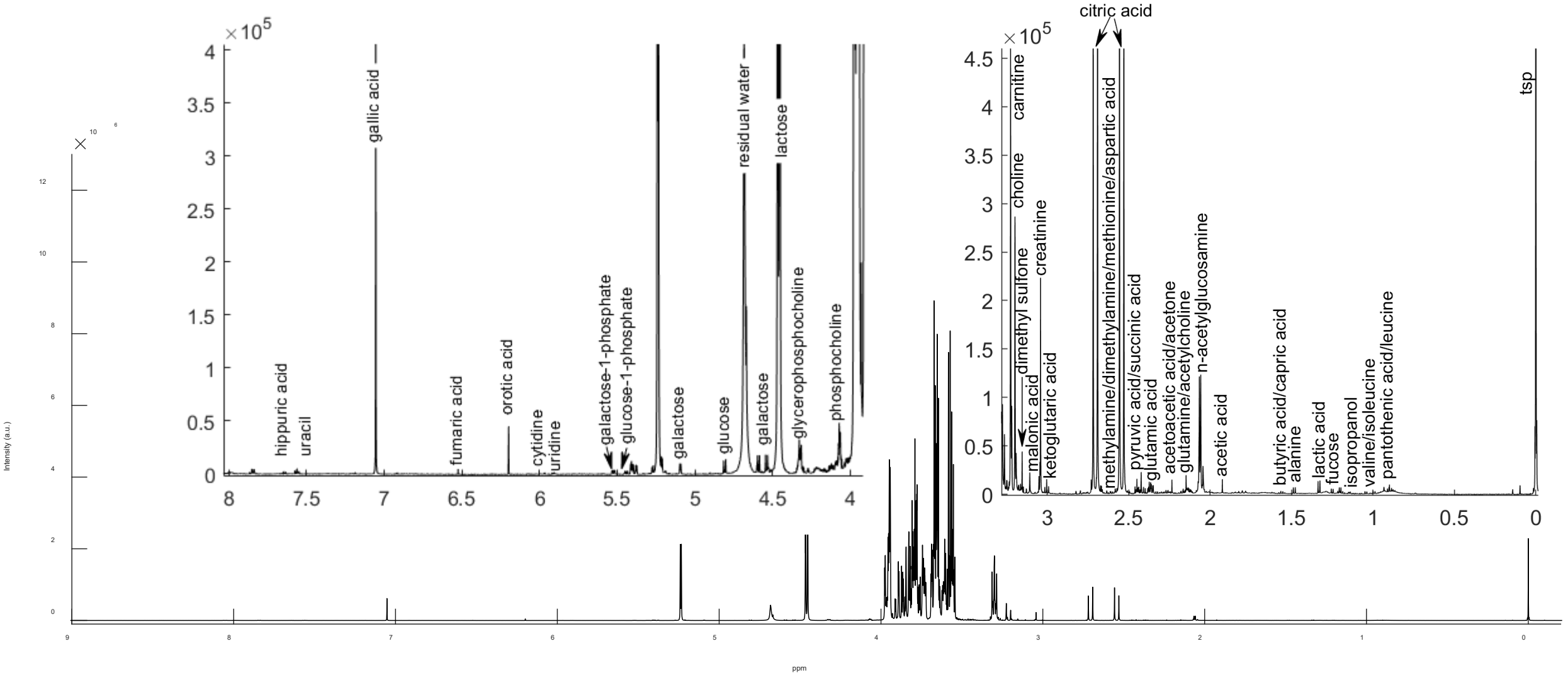
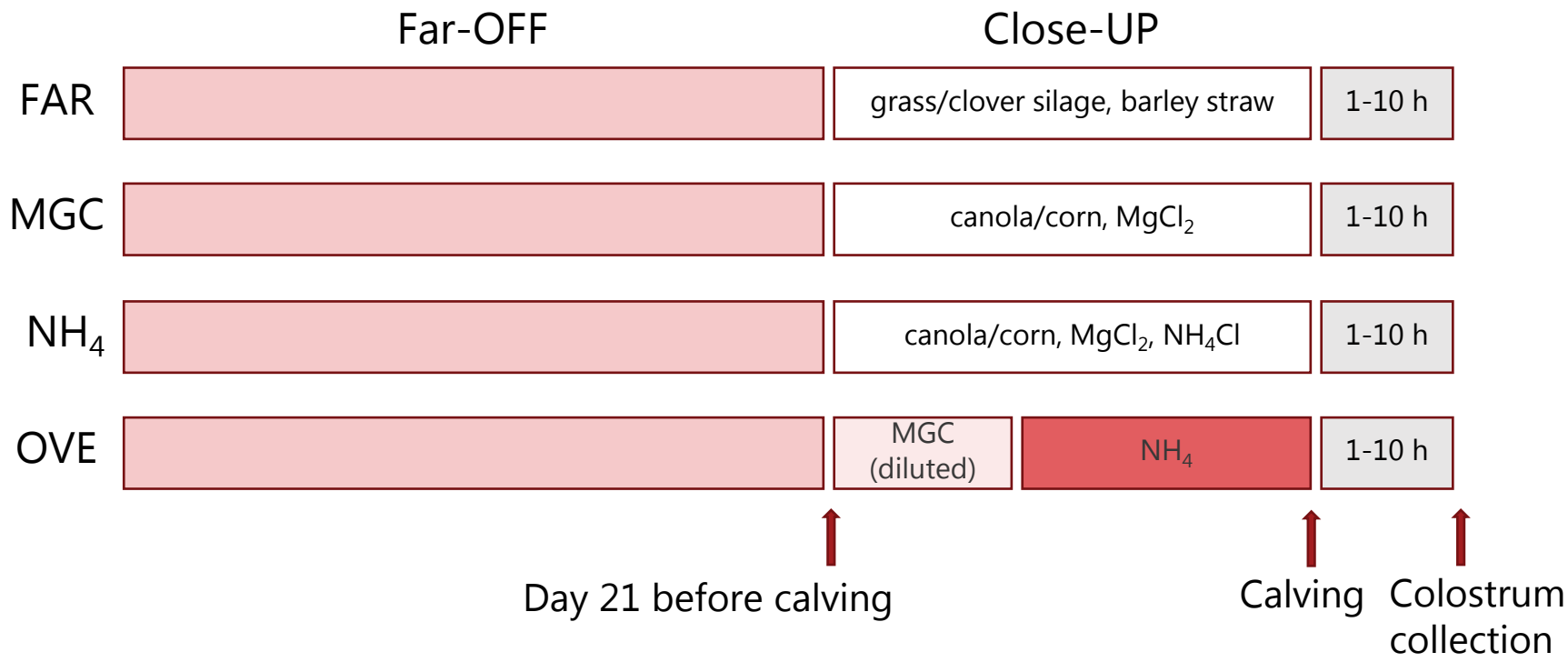


Table of identified metabolites

	Interval name	Chemical shift (ppm)	Multiplicity	Type of protons
1	pantothenic acid	0.95	s	CH_3
2	leucine	0.97	t	CH_3
3	valine	0.99	s	CH_3
4	isoleucine	1.02	d	CH_3
5	fucose	1.26	d	CH_3
6	lactic acid	1.34	d	CH_3
7	alanine	1.49	d	CH_3
8	acetic acid	1.93	s	CH_3
9	n-acetyl glucosamine	2.06	s	CH_2
10	acetylcholine	2.15	s	CH_3
11	acetoacetic acid	2.24	s	CH_3
12	malic acid	2.36	d	CH_3
13	carnitine	2.42	s	CH_2
14	dimethylamine	2.50	s	CH_2
15	citrate	2.55	d	$2 \times -CH_2$
16	methylamine	2.60	s	CH_2
17	aspartic acid	2.68	dd	CH_3
		…		
39	cytidine	6.07	d	-CH
40	orotic acid	6.20	s	-CH
41	fumaric acid	6.53	s	-CH
42	tyrosine	6.91	d	$2 \times$ -CH
43	uracil	7.06	d	N-CH
44	tryptophan	7.54	d	N-CH
45	hippurate	7.56	m	-CH, $2 \times CH_2$
46	uridine	7.64	t	-CH
47	formic acid	7.86	s	-CH
		18 unknowns		
		9 BINS		

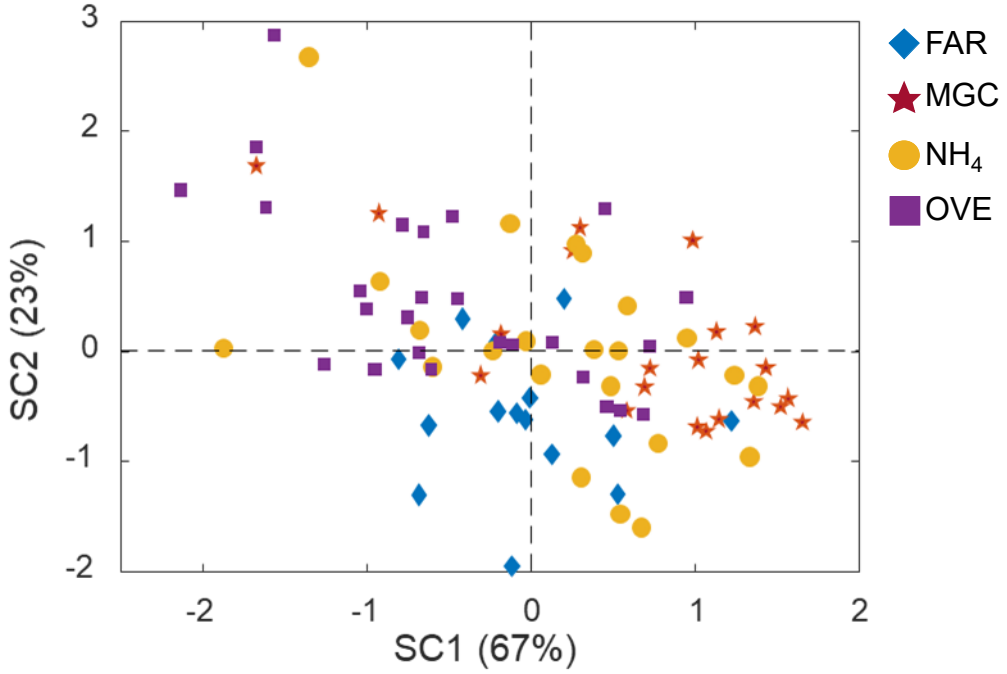
Colostrum samples design – 2021 (104 samples)

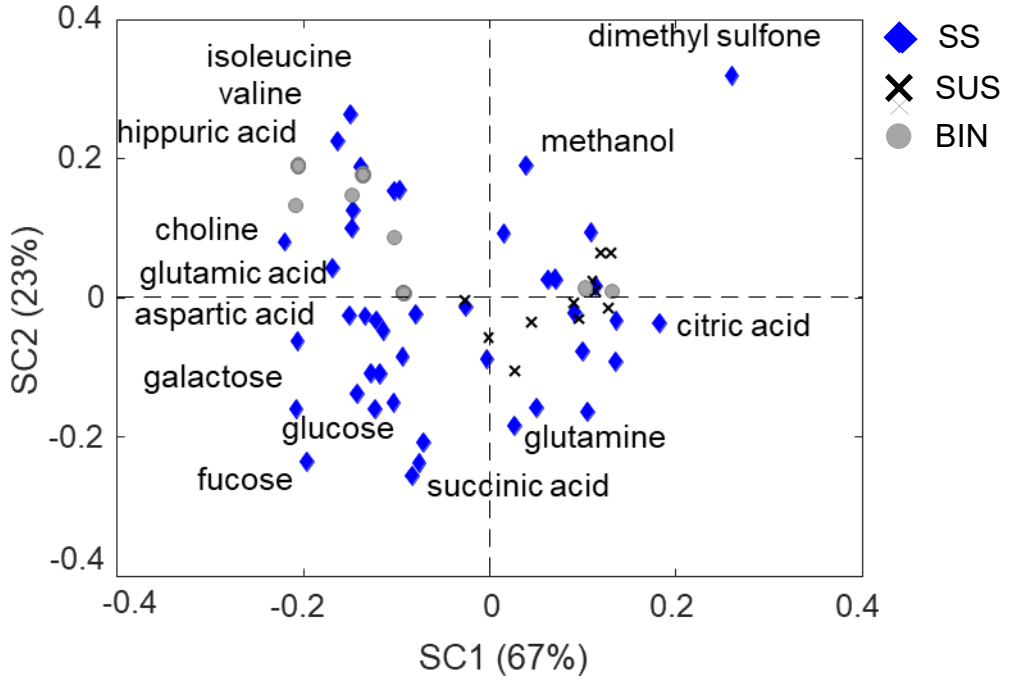


FAR: Far-OFF, MGC: magnesium chloride, NH_4: ammonium, OVE: overfeeding

Effect of different feedings on the colostrum metabolome

- ANOVA – simultaneous components analysis: p-value=0.1, accounts for 4.5% of the total variation

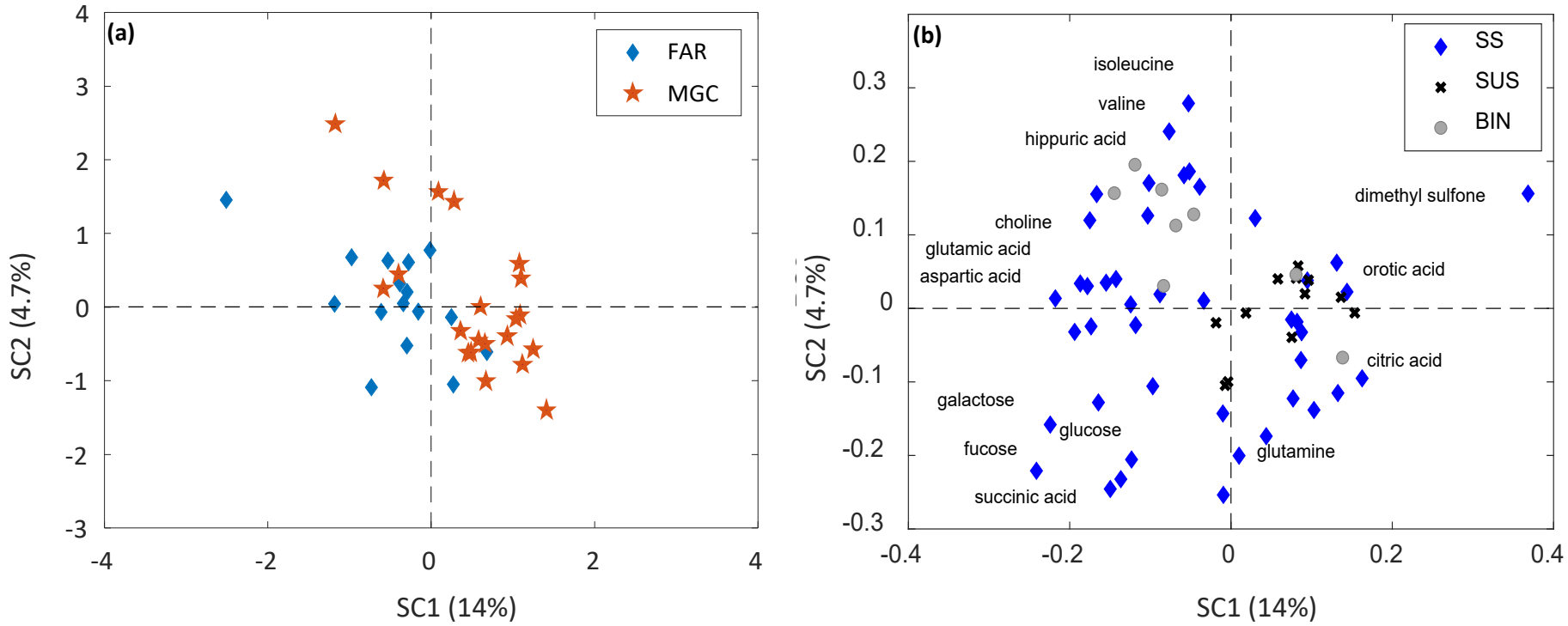




- ✓ Partial separation due to dimethyl sulfone, methanol, isoleucine, valine, fucose, glucose and succinic acid

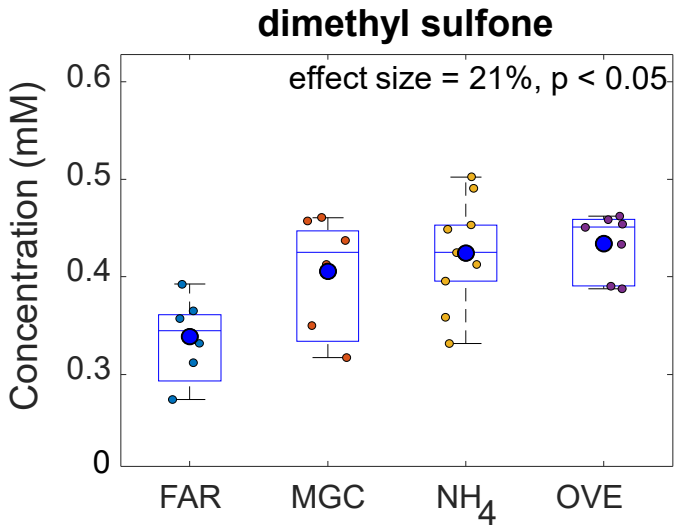
Effect of different feedings on the colostrum metabolome

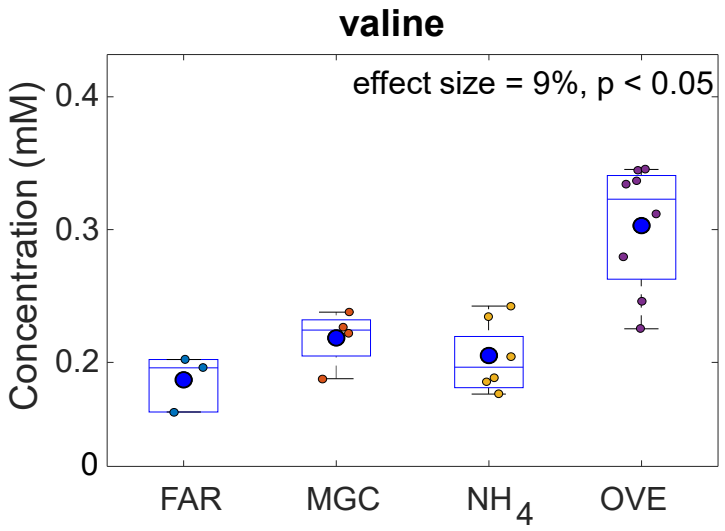
- ANOVA – simultaneous components analysis: p-value=0.04, accounts for 4.9% of the total variation

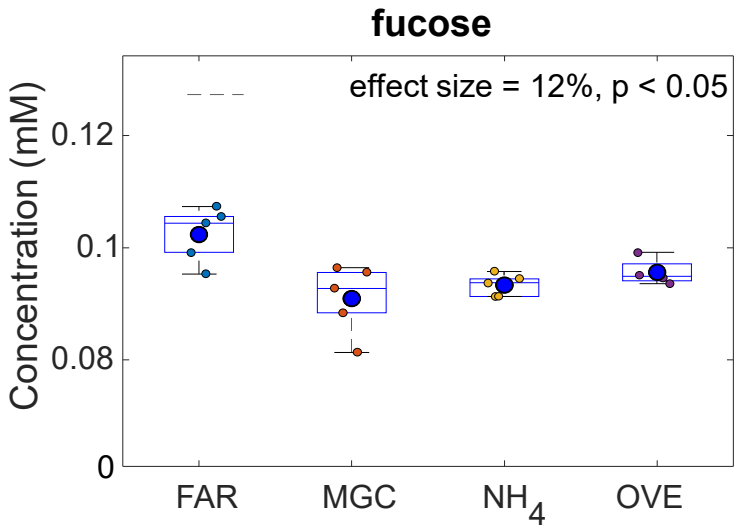


- ✓ Partial separation due to dimethyl sulfone, isoleucine, valine, fucose, glucose and succinic acid

Effect of different feedings on the colostrum metabolome







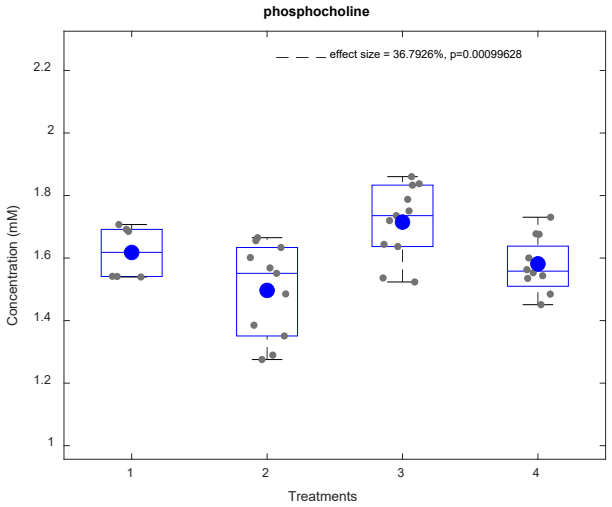
✓ Dimethyl sulfone, valine and fucose discriminate the colostrum from cows following different feedings

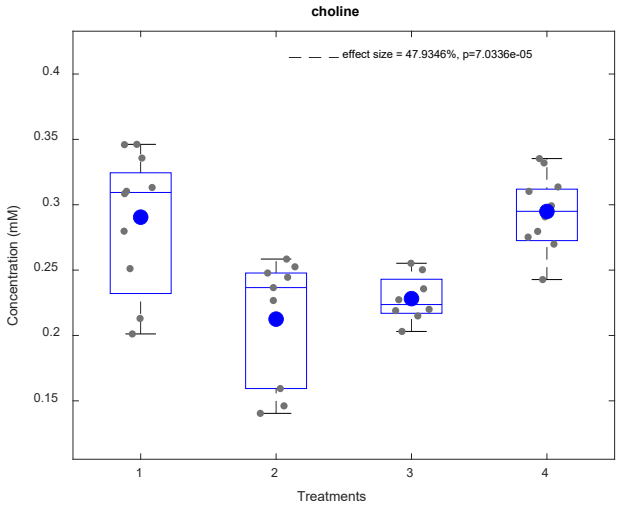
Discriminant metabolites

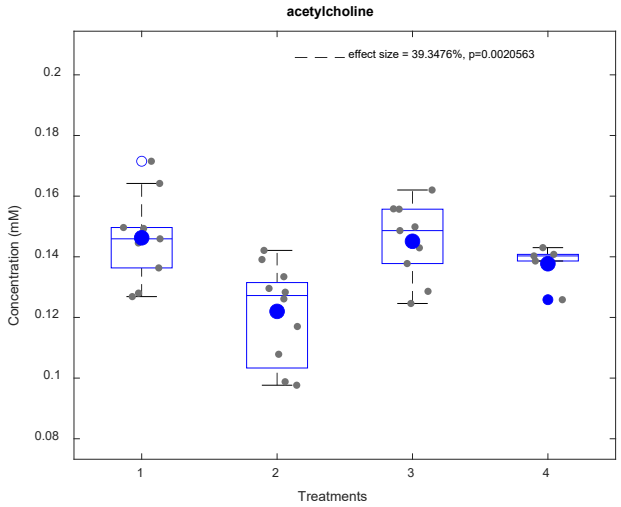
- 31 out of 47 show significant differences among different groups

Discriminant metabolites

Metabolites associated to de novo synthesis of phosphatidylcholine

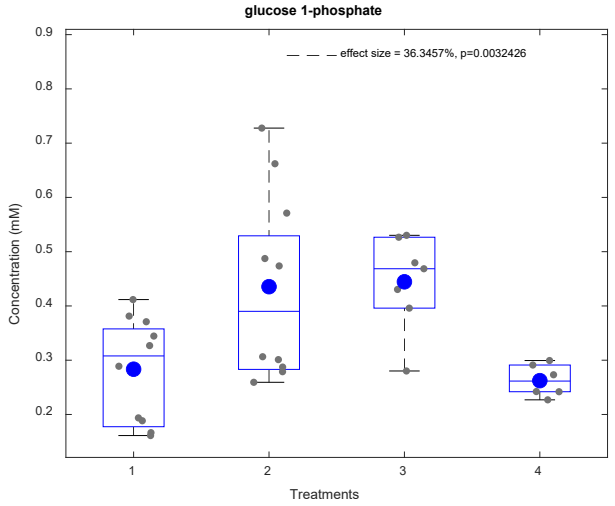


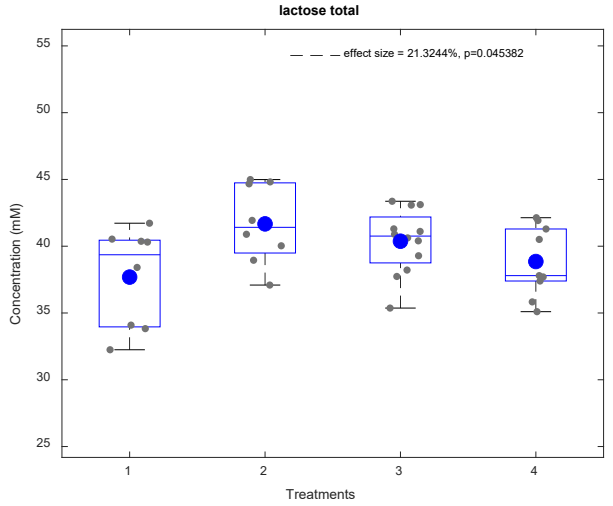


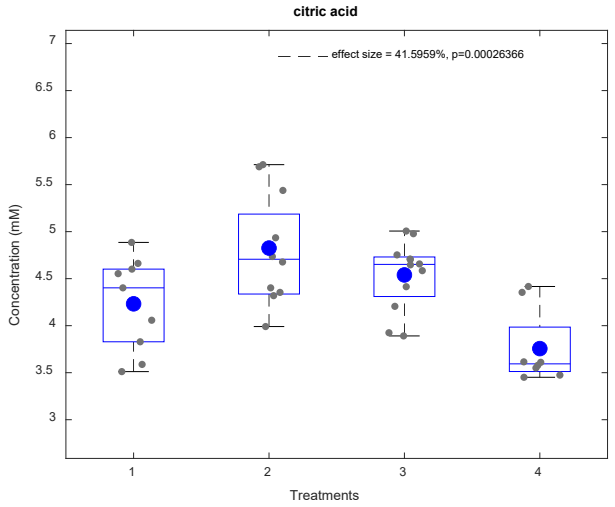


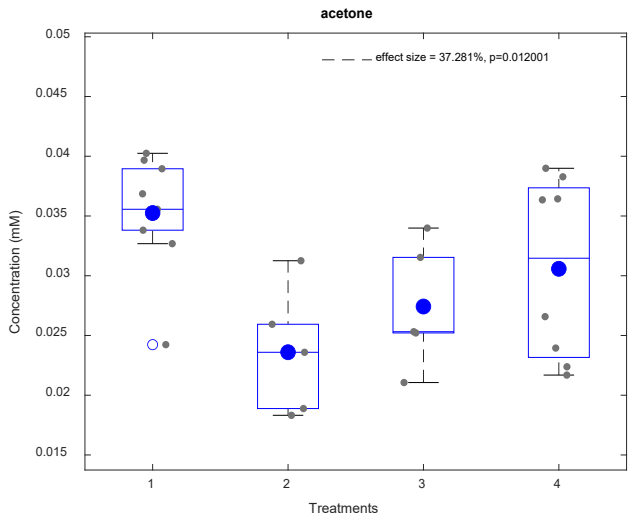
✓ Strongly correlated to apoptotic cells

Discriminant metabolites

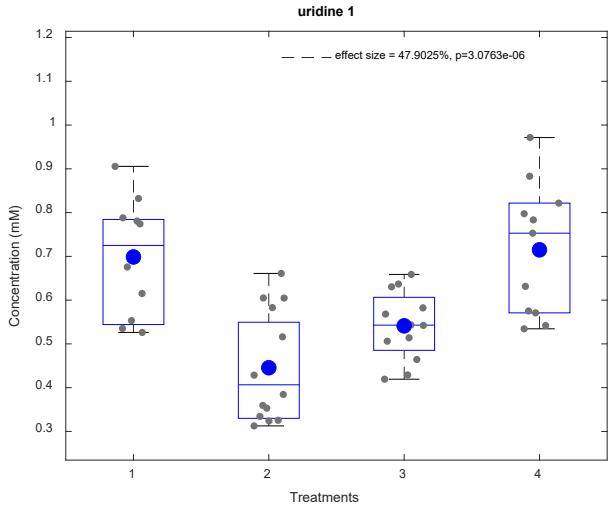


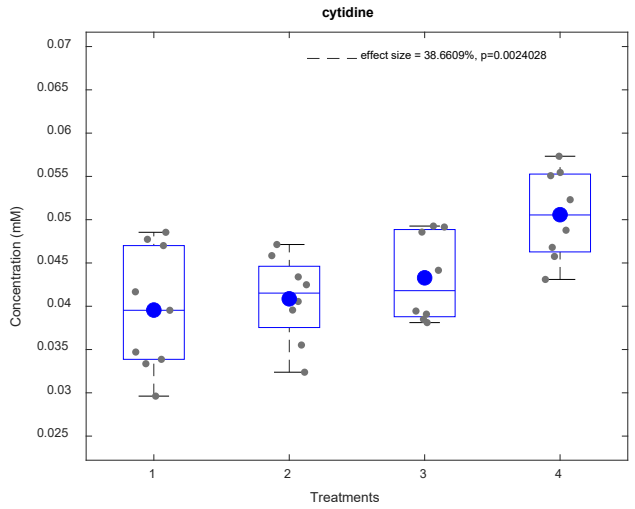






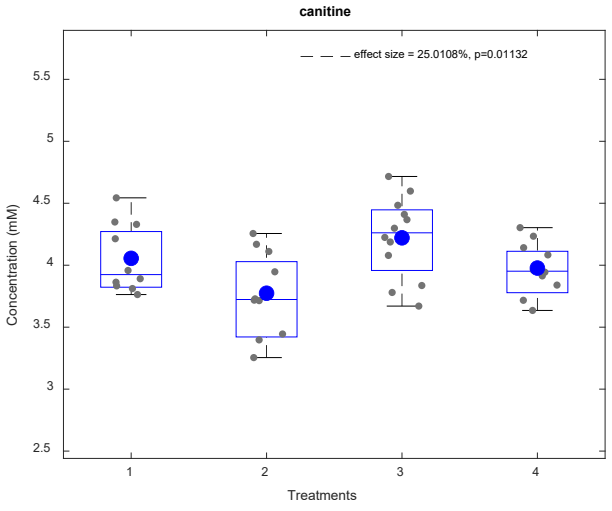
Discriminant metabolites

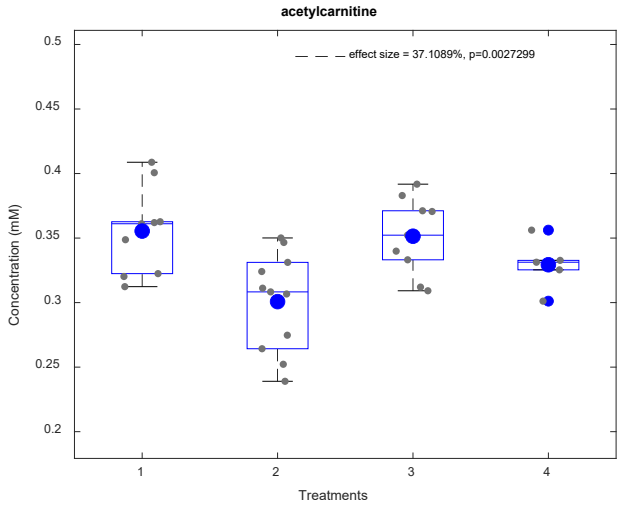




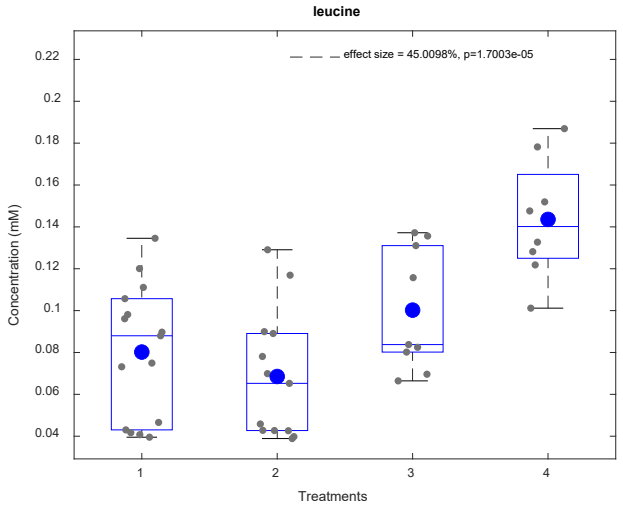
Discriminant metabolites

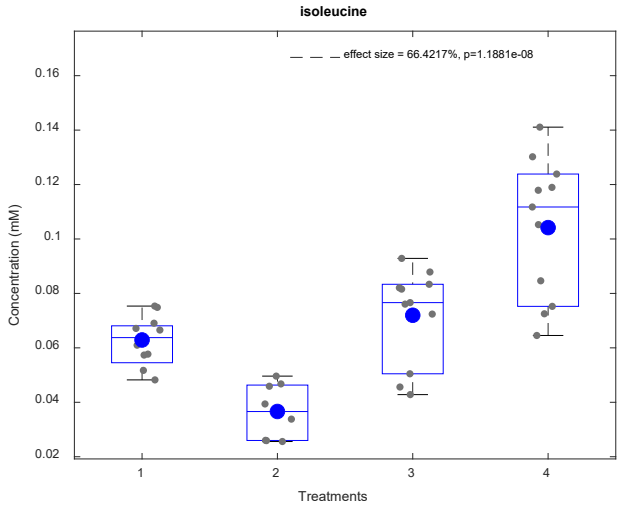
Metabolites associated to fat metabolism

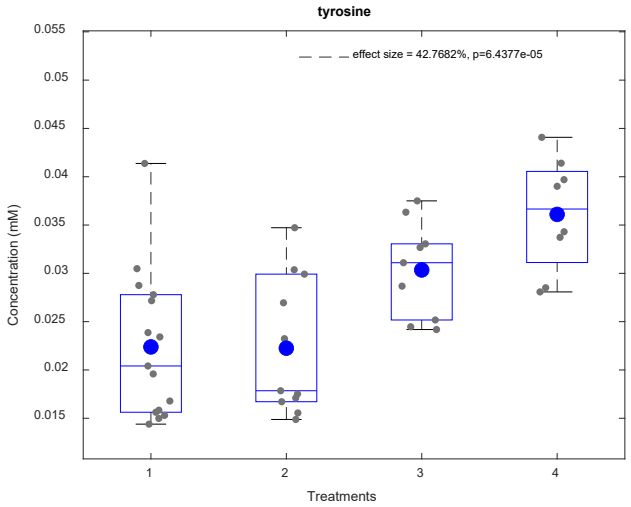


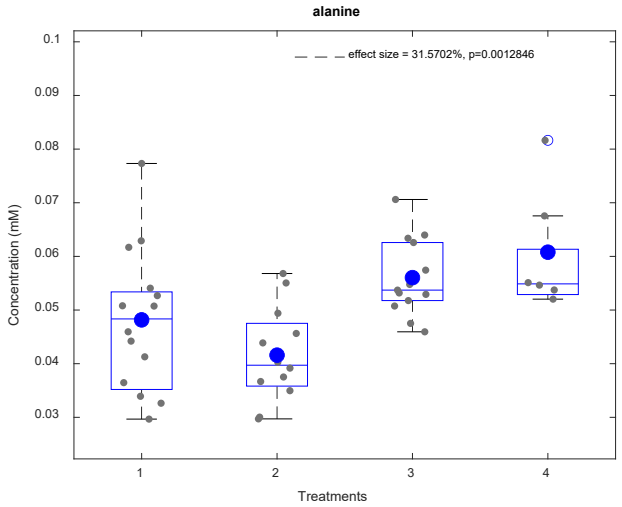


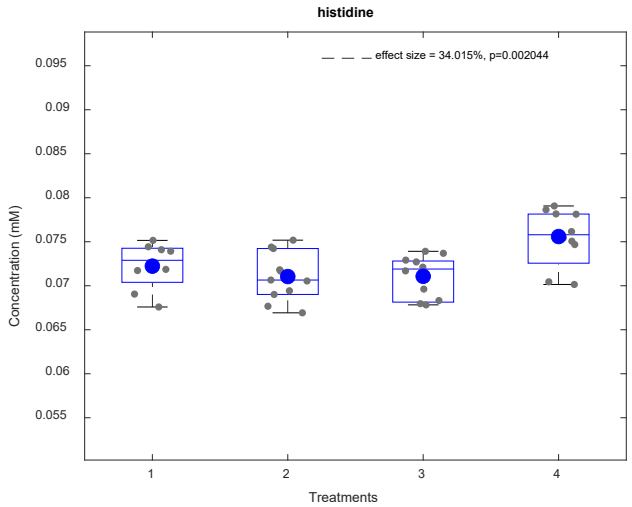
Discriminant metabolites

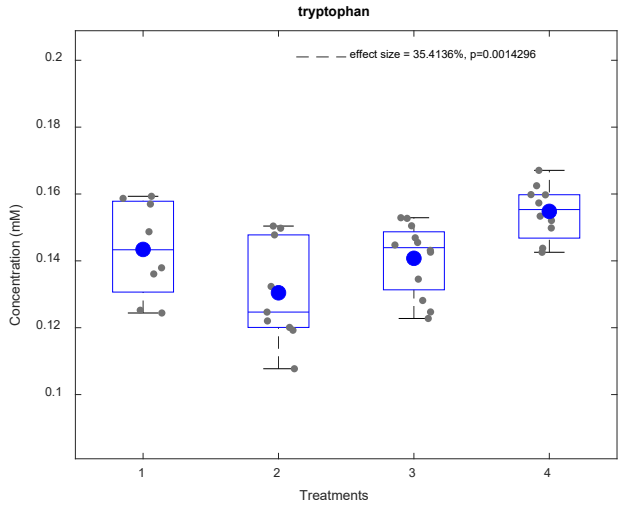


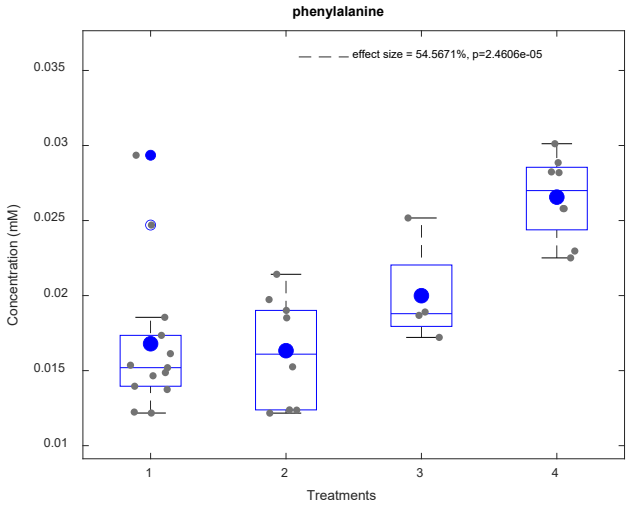


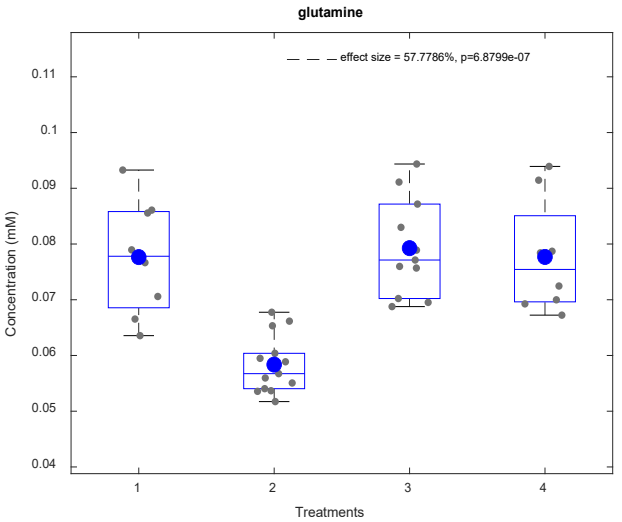




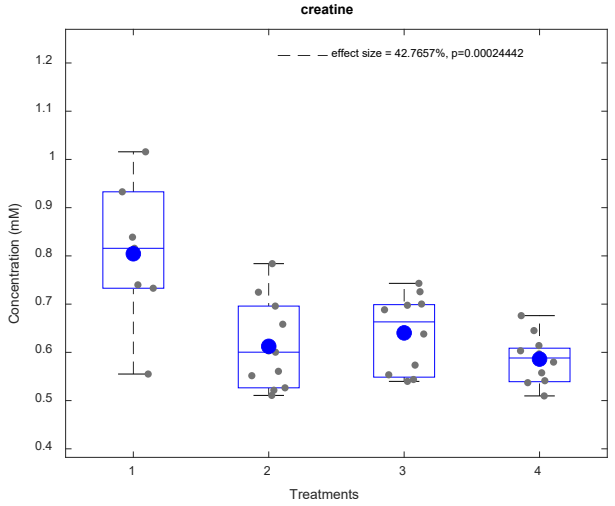


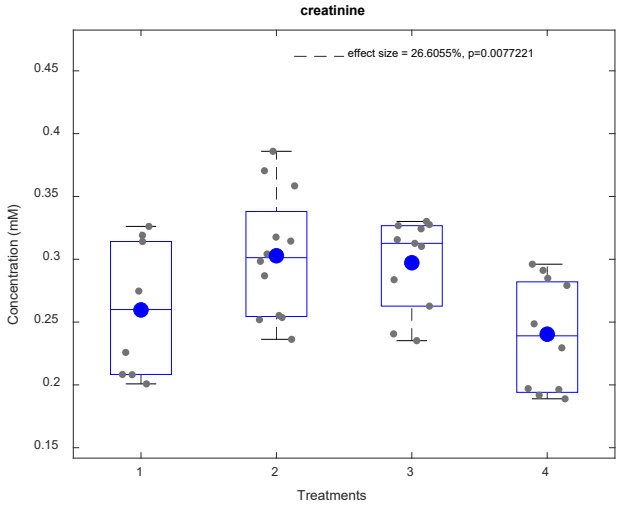


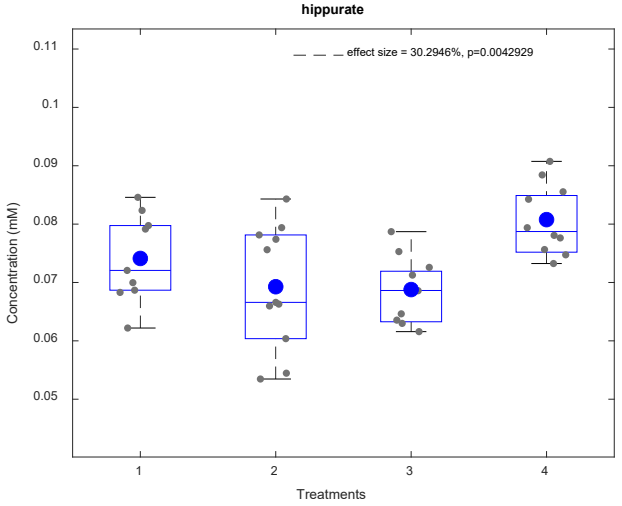


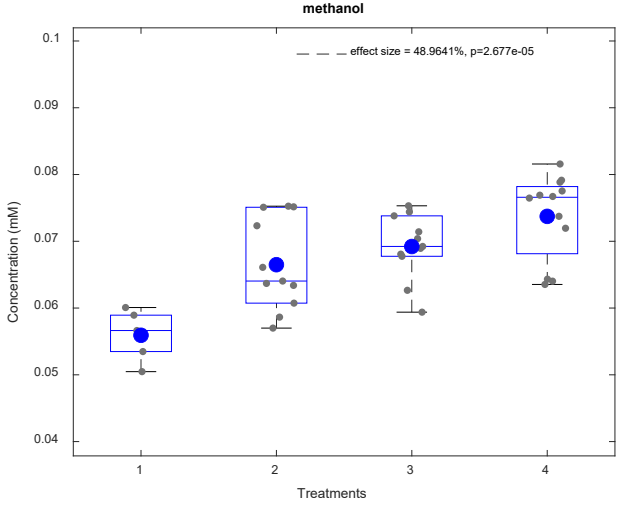


Discriminant metabolites

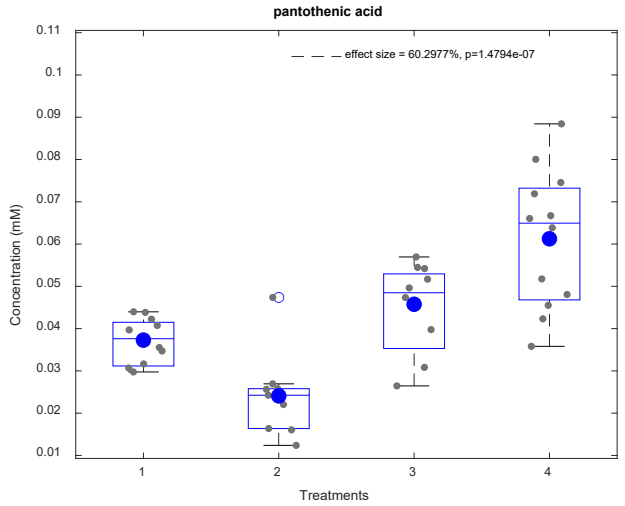


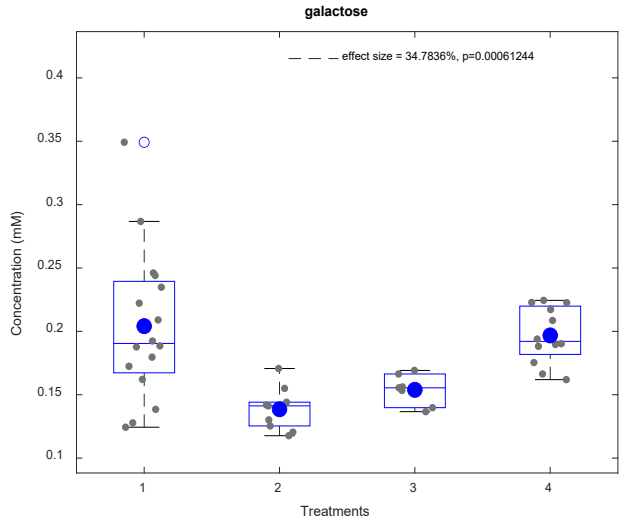


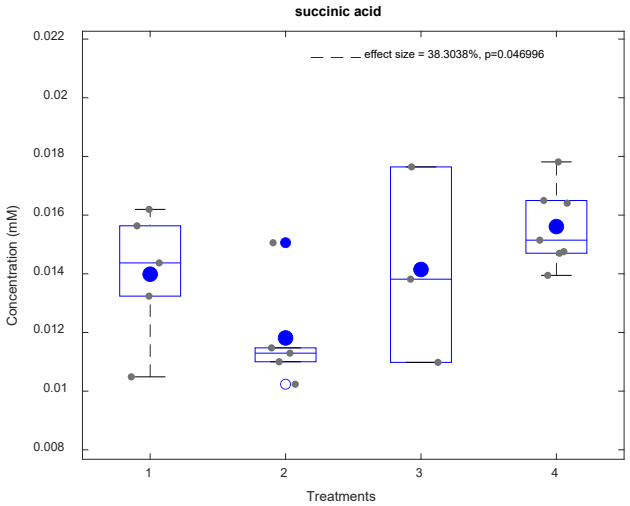


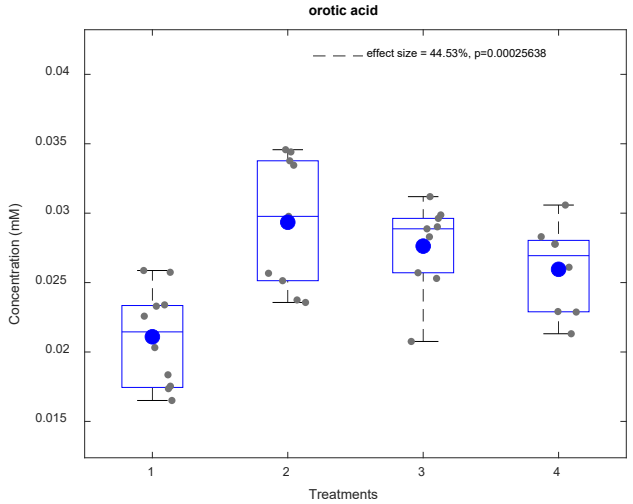


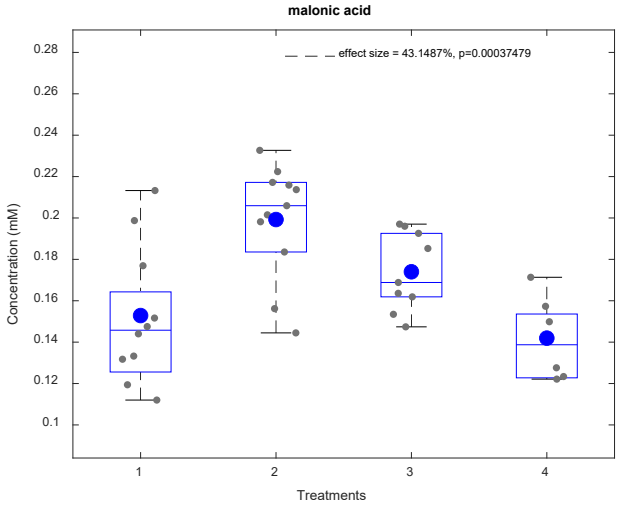
Discriminant metabolites



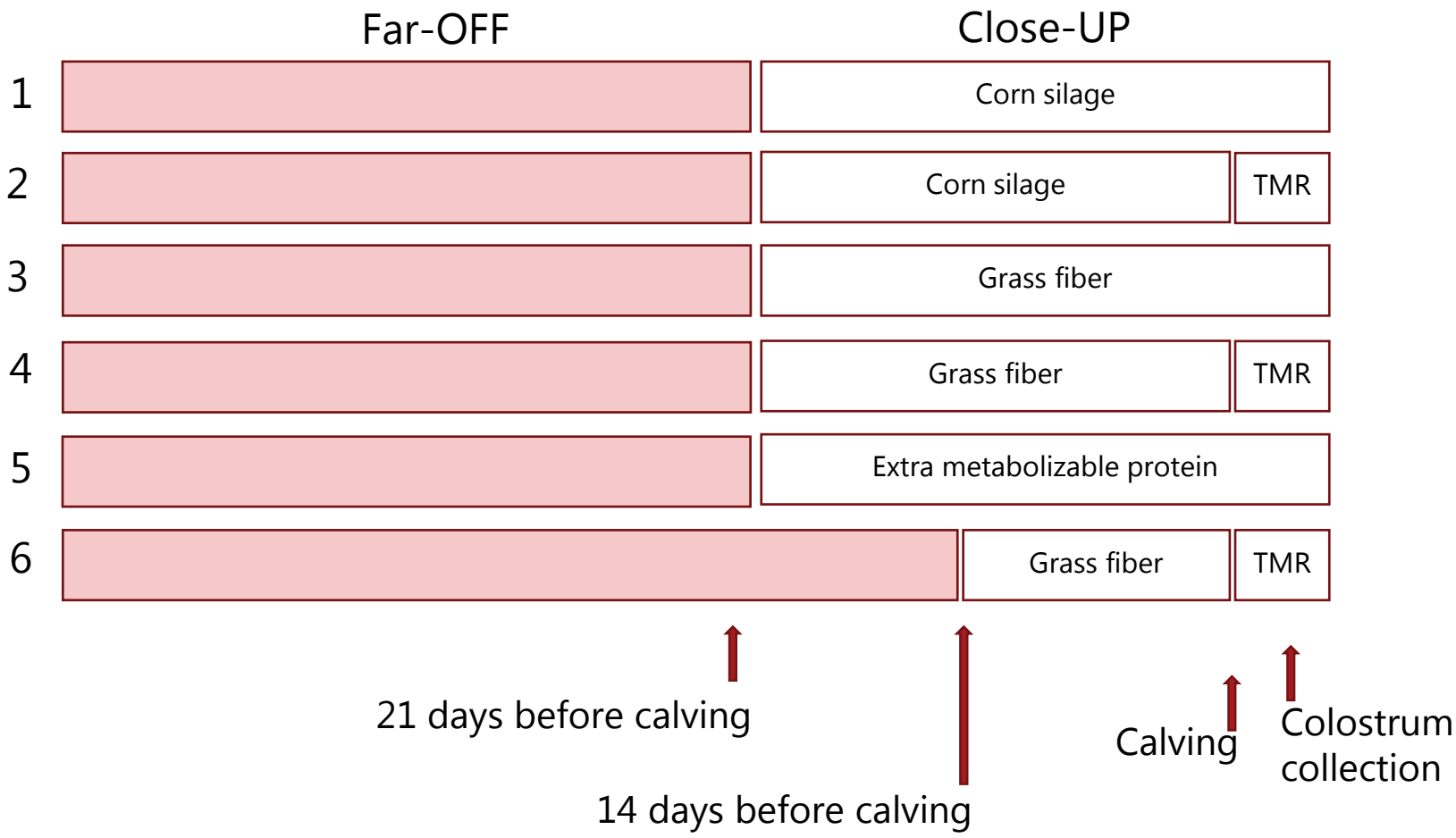








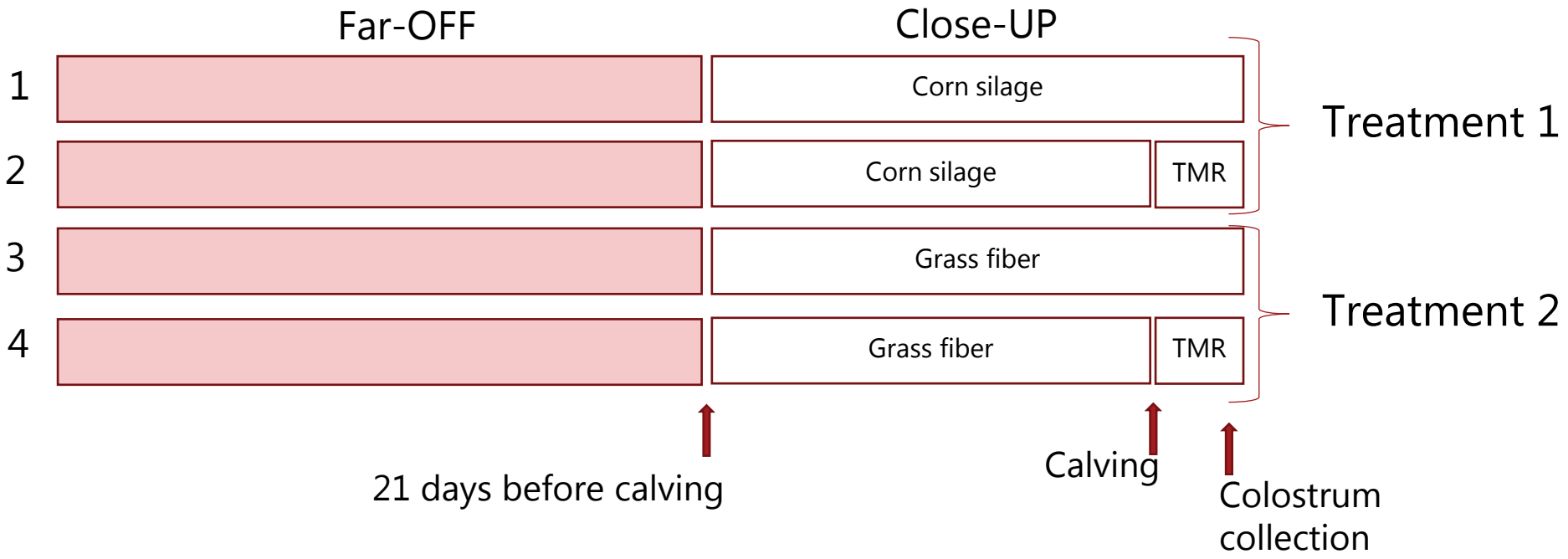
Colostrum samples design – 2022 (143 samples)



Samples

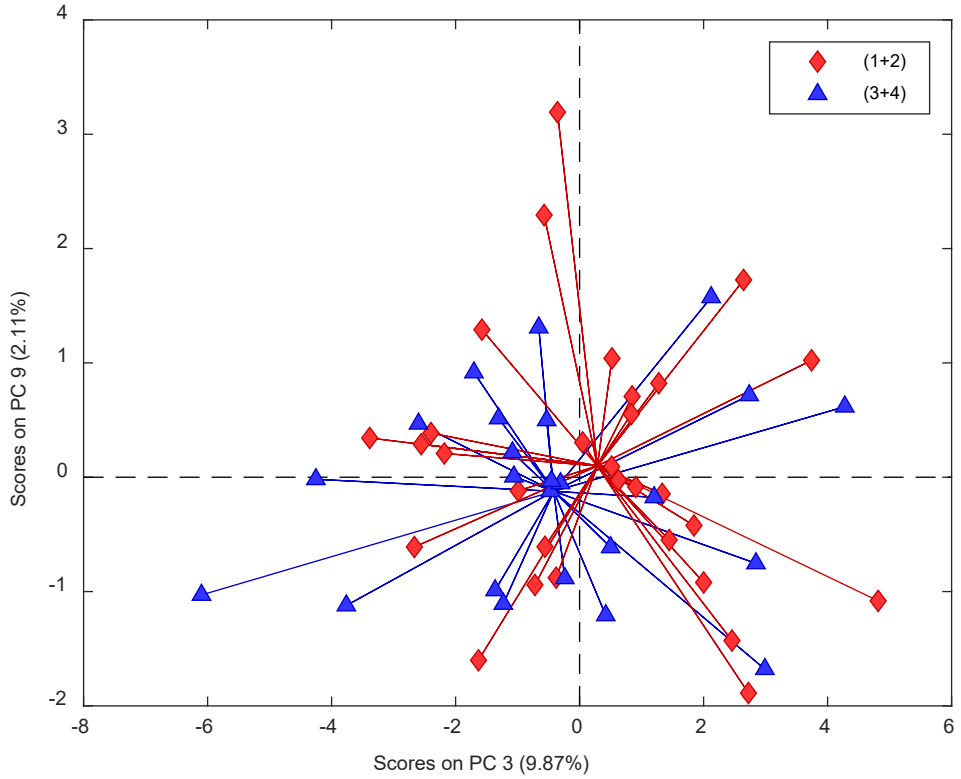
- 13 samples were not analysed due to coagulation

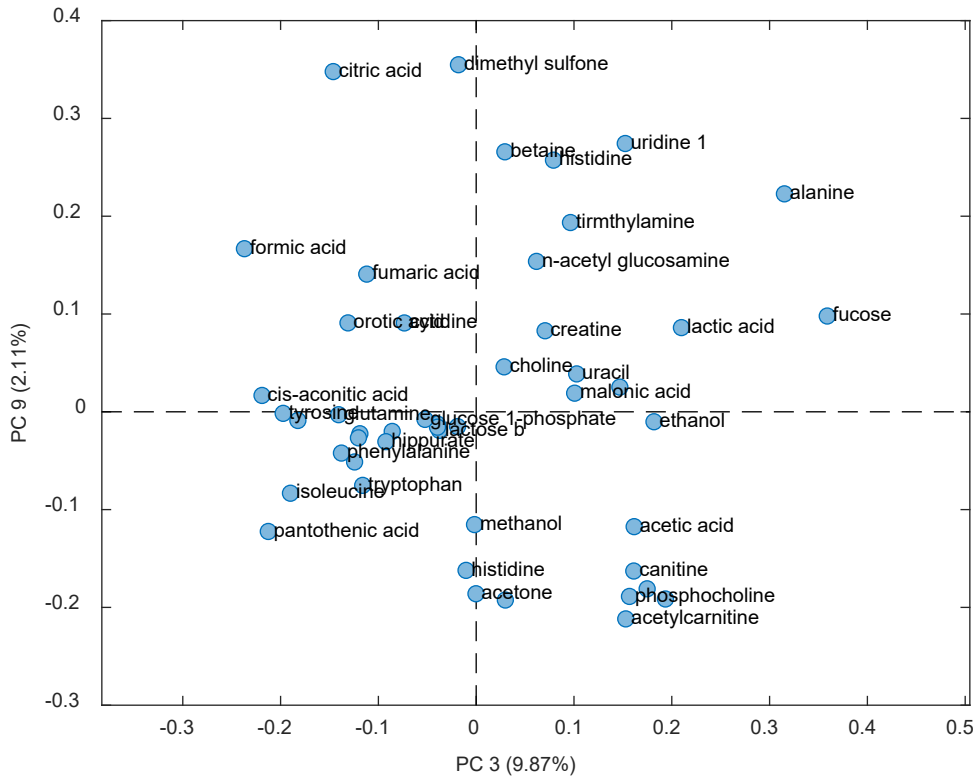
Study design – 2022a



Effect of different feedings on the colostrum metabolome

- ANOVA – simultaneous components analysis: **p-value=0.5**

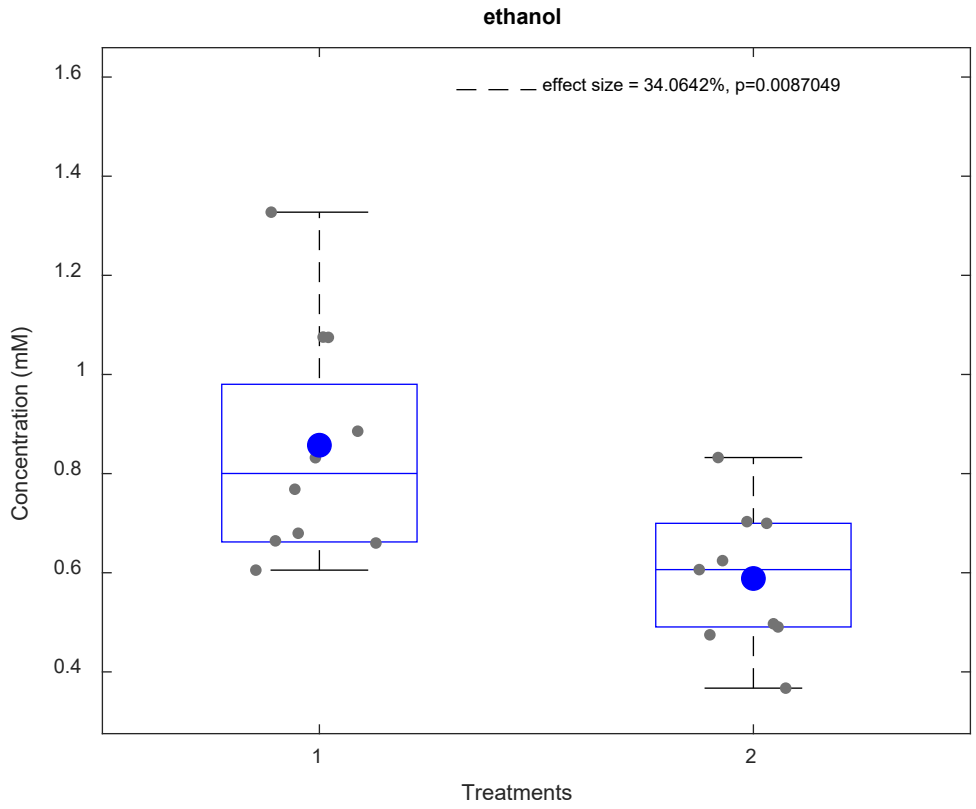


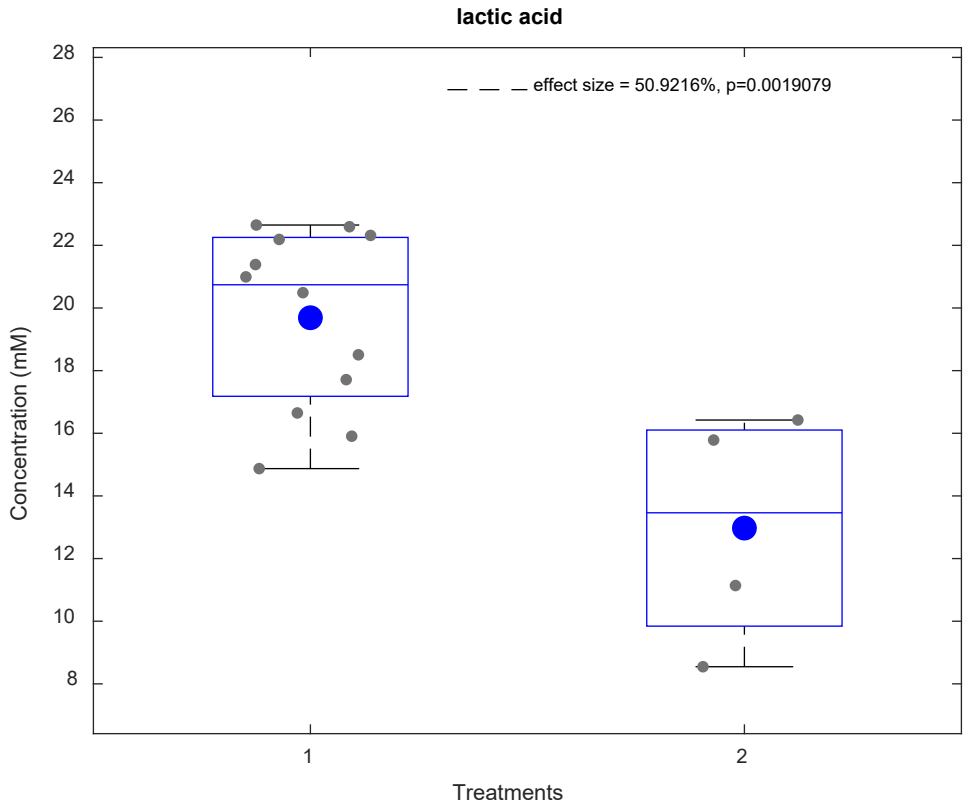


Discriminant metabolites

- 12 out of 47 show significant differences among different groups

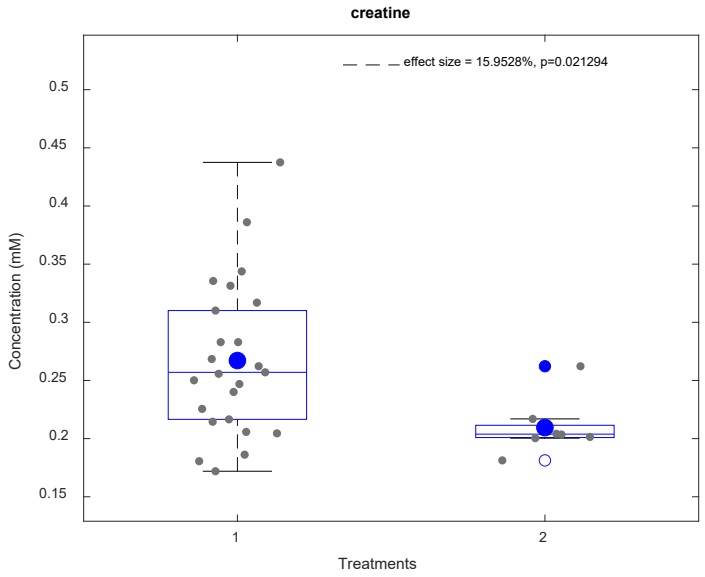
Discriminant metabolites

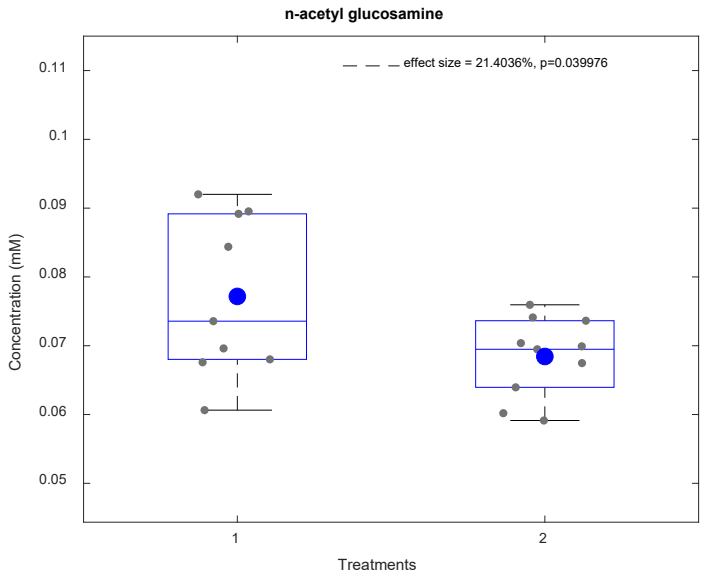


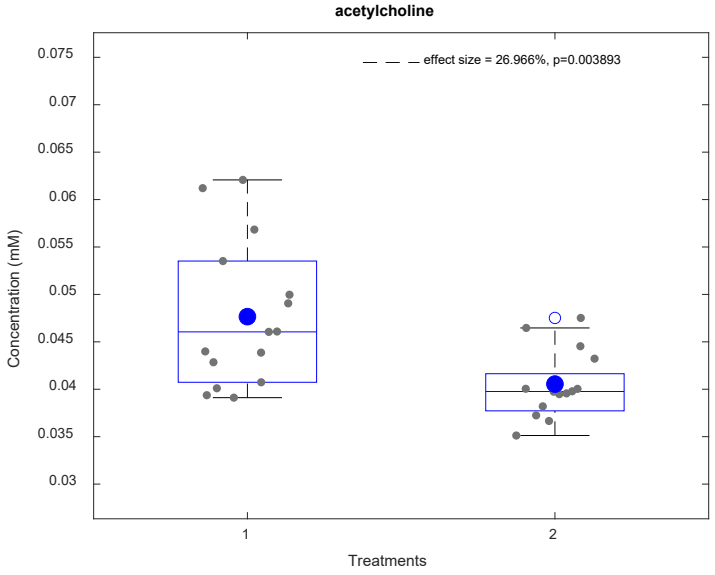


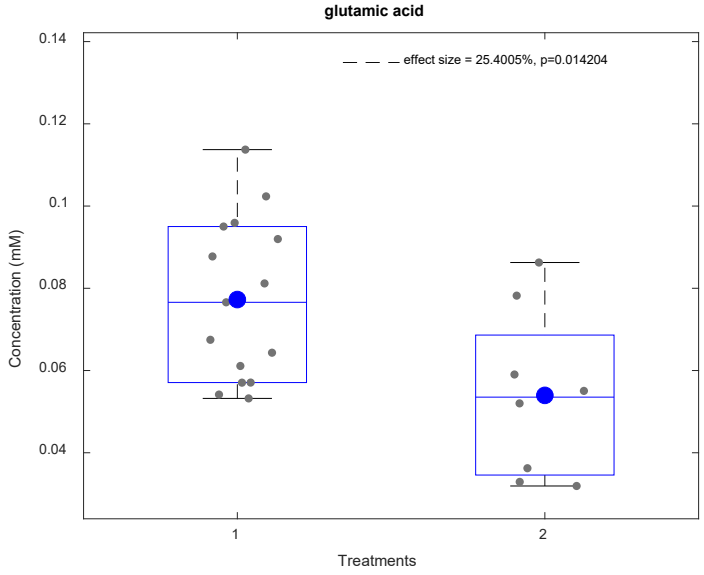
✓ microbial activity

Discriminant metabolites

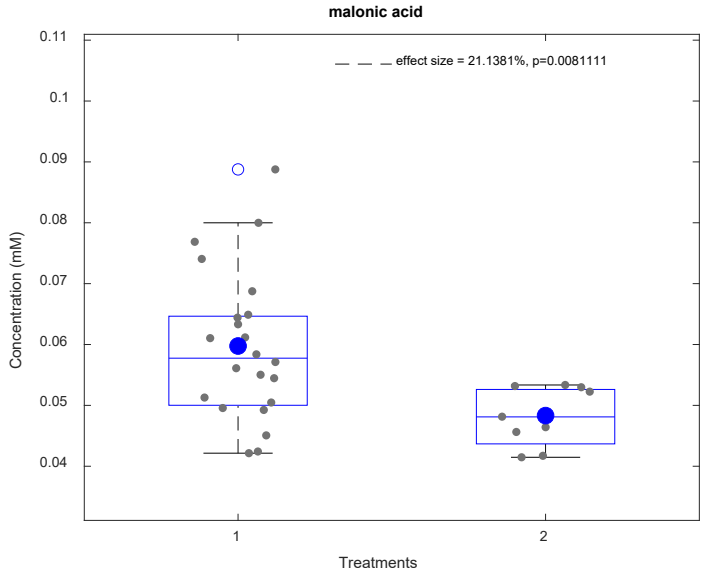


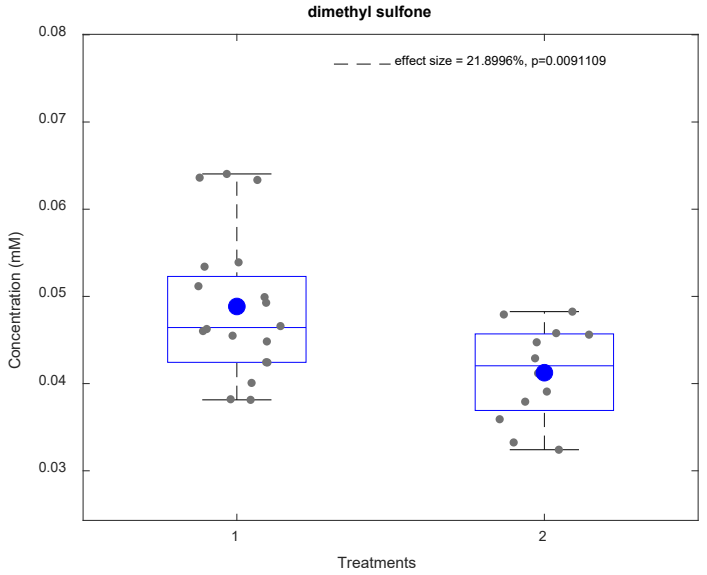


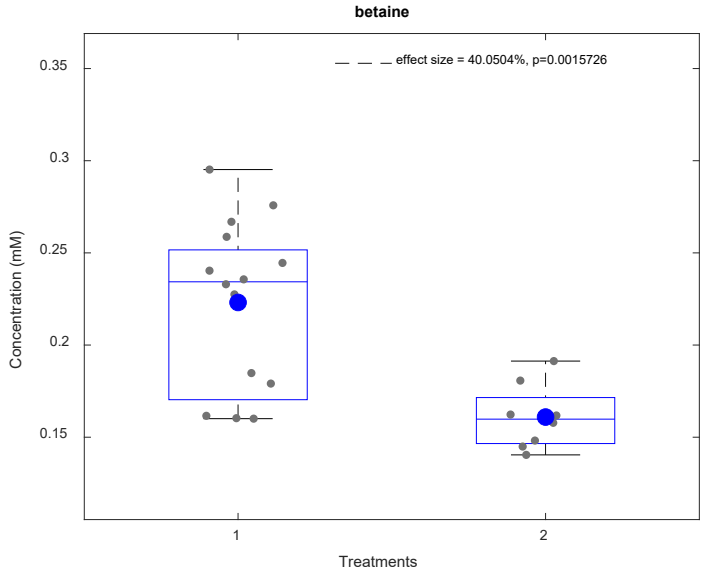


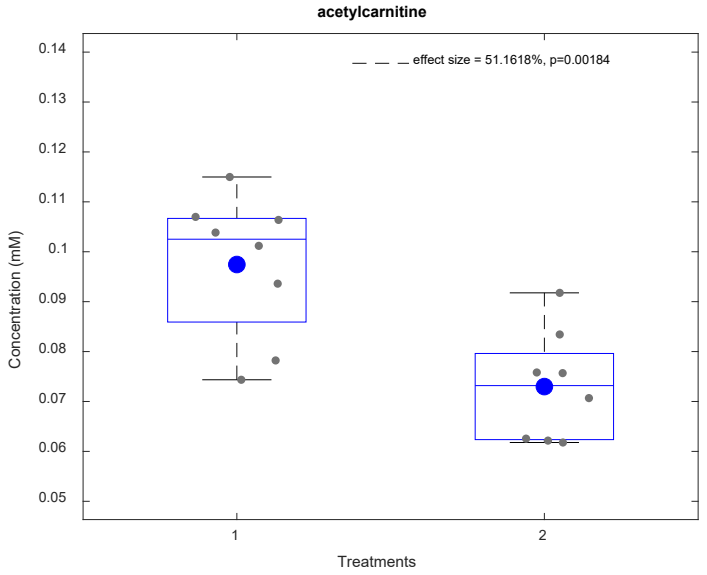


Discriminant metabolites

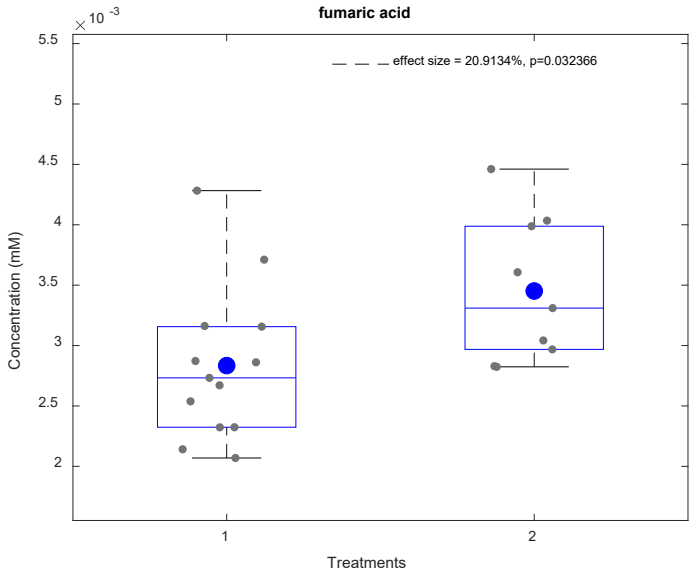


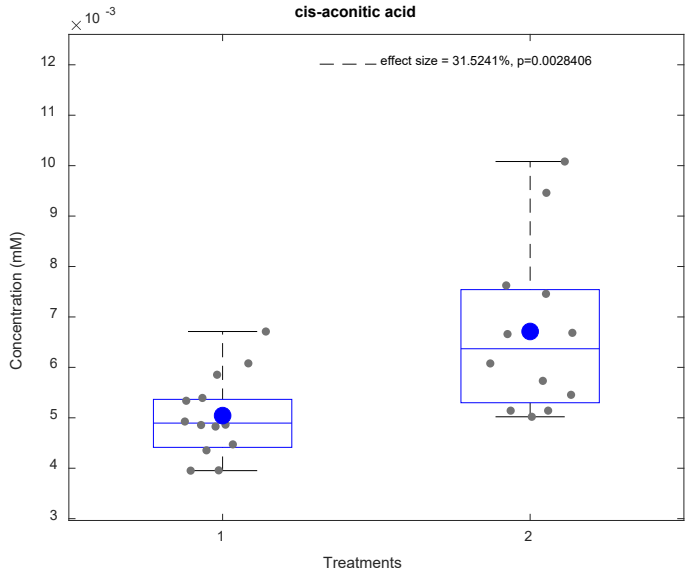




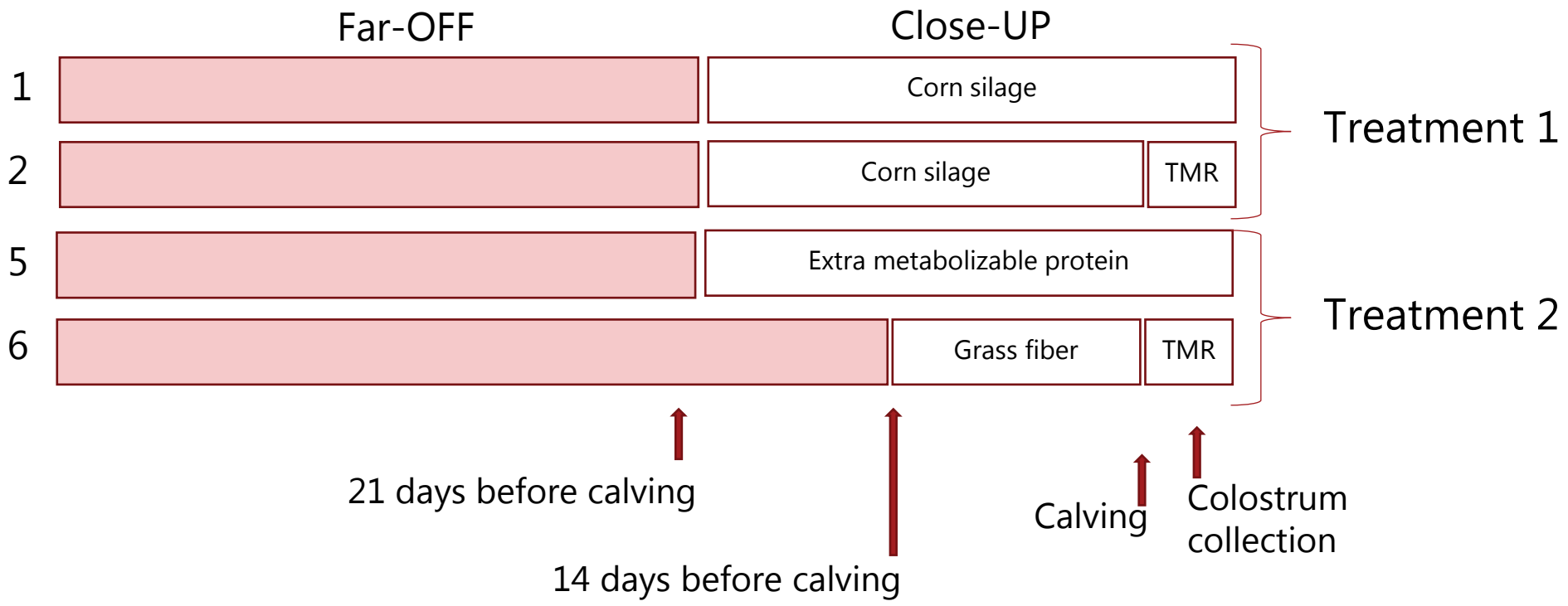


Discriminant metabolites



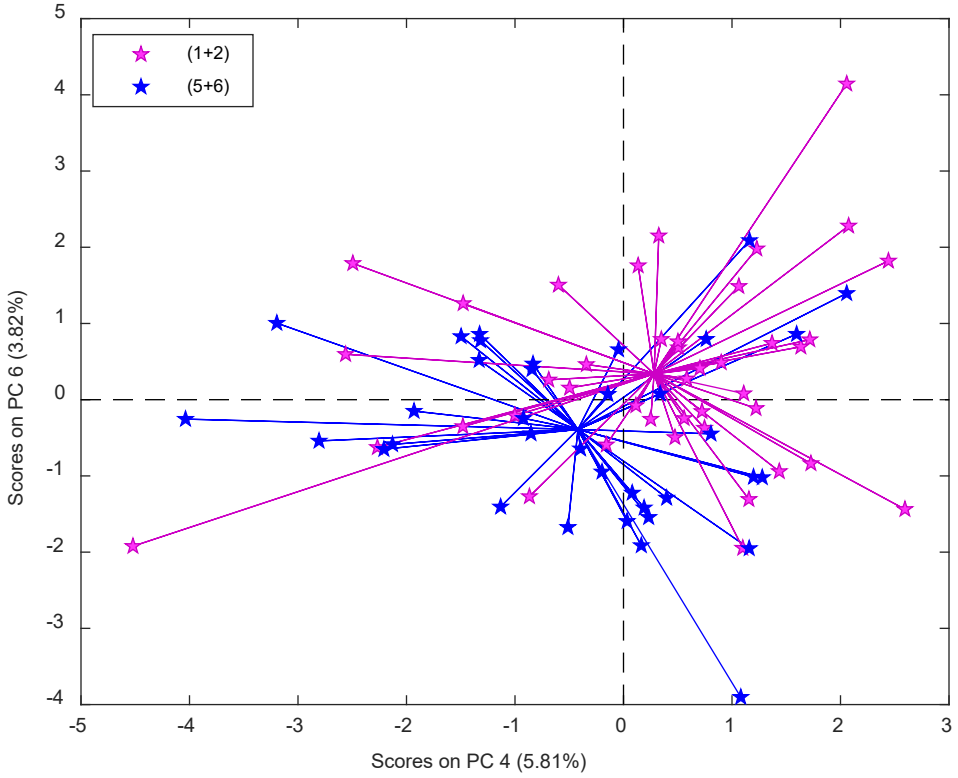


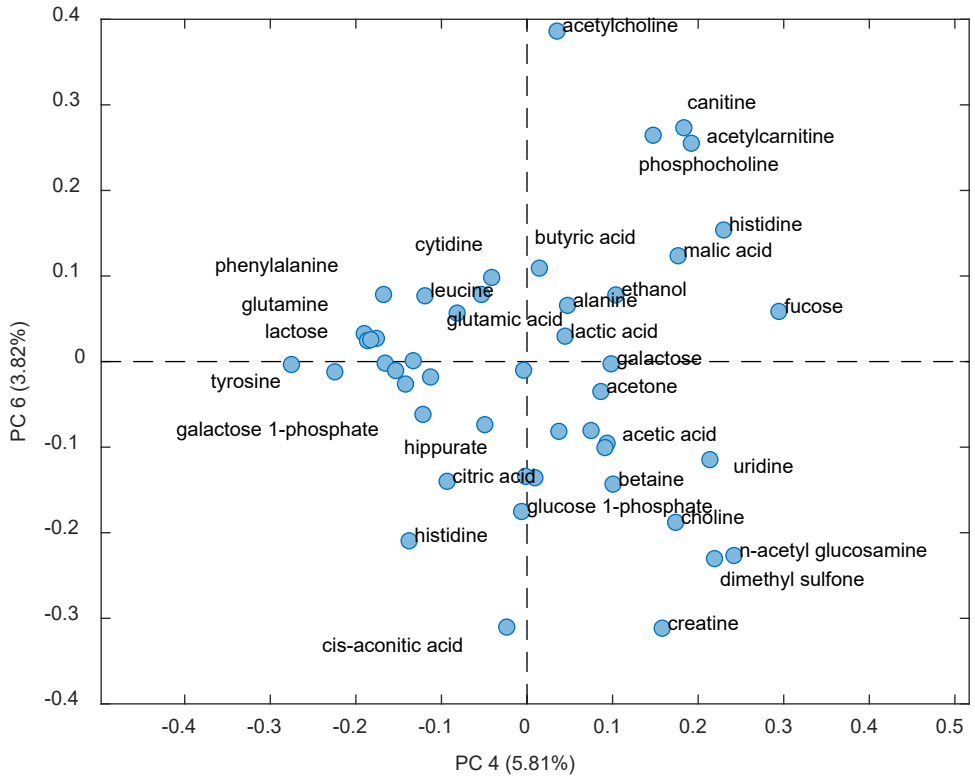
Study design – 2022b



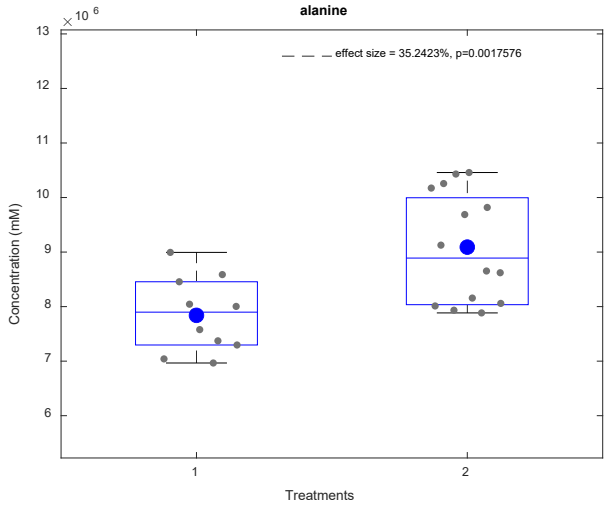
Effect of different feedings on the colostrum metabolome

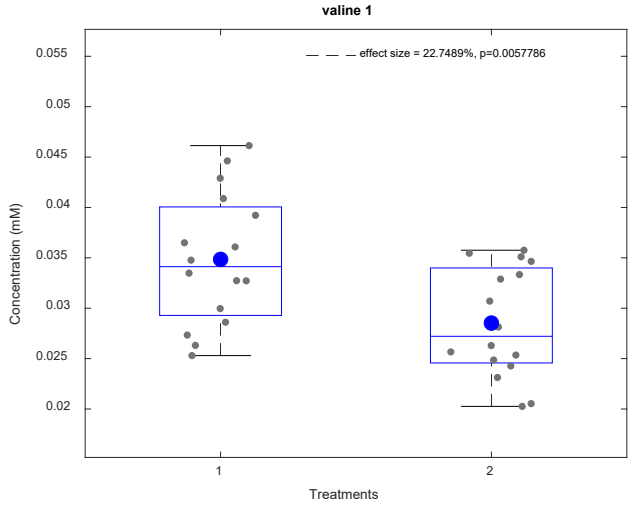
- ANOVA – simultaneous components analysis: **p-value=0.7**

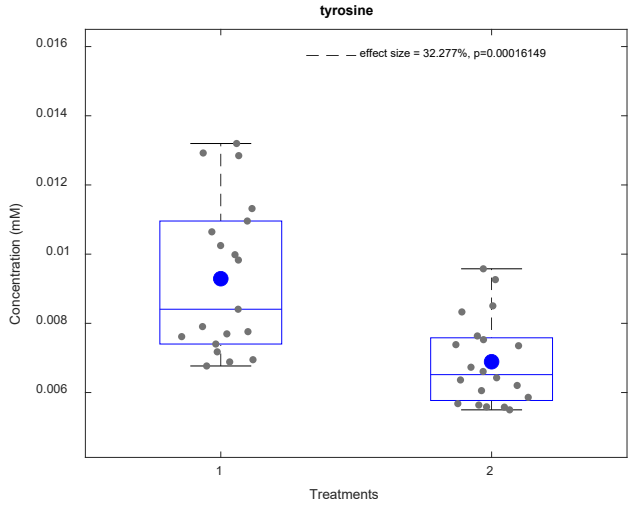


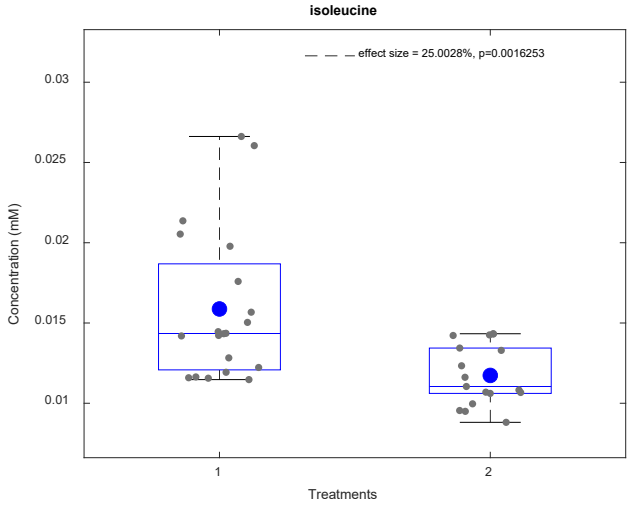


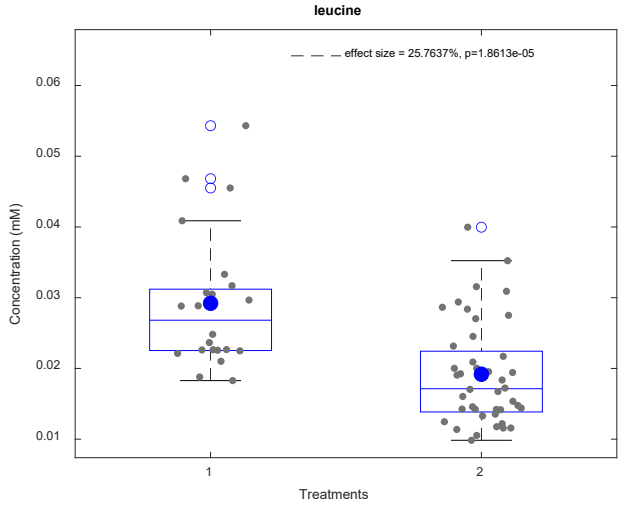
Discriminant metabolites

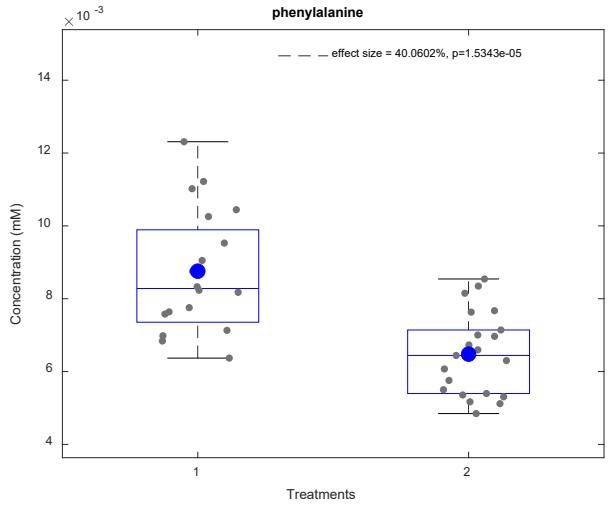




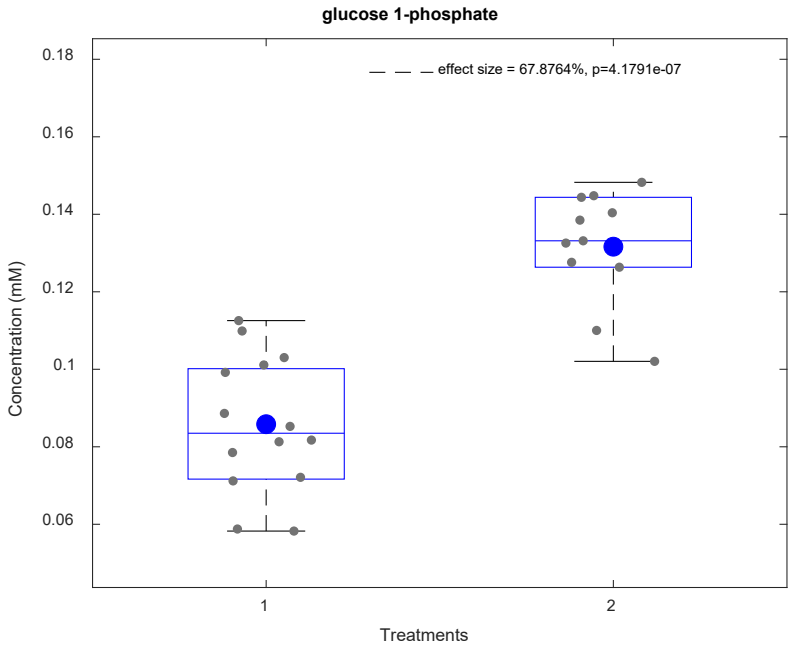


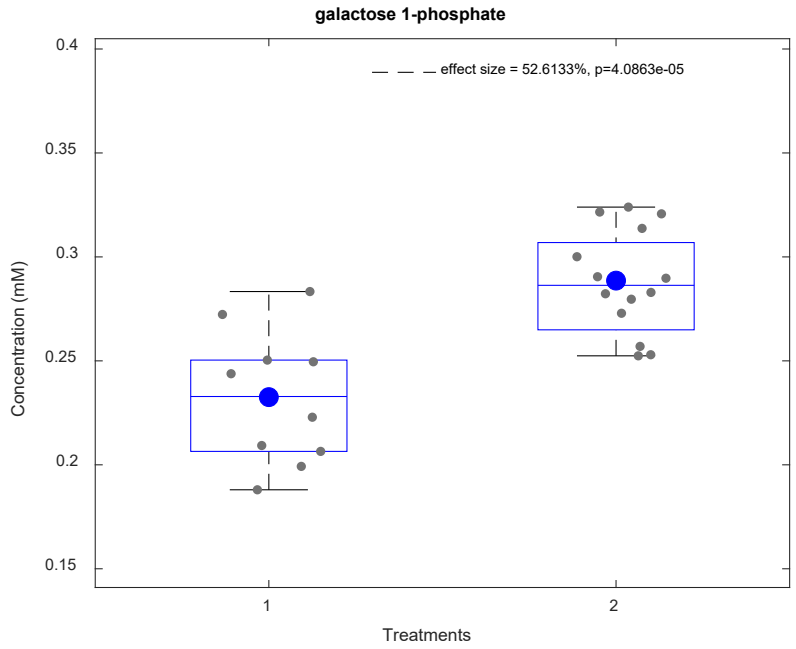


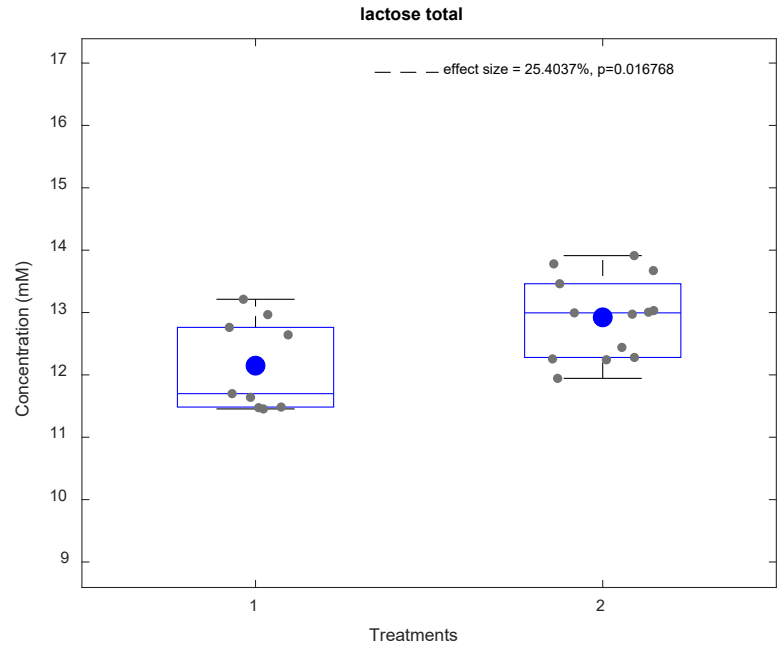




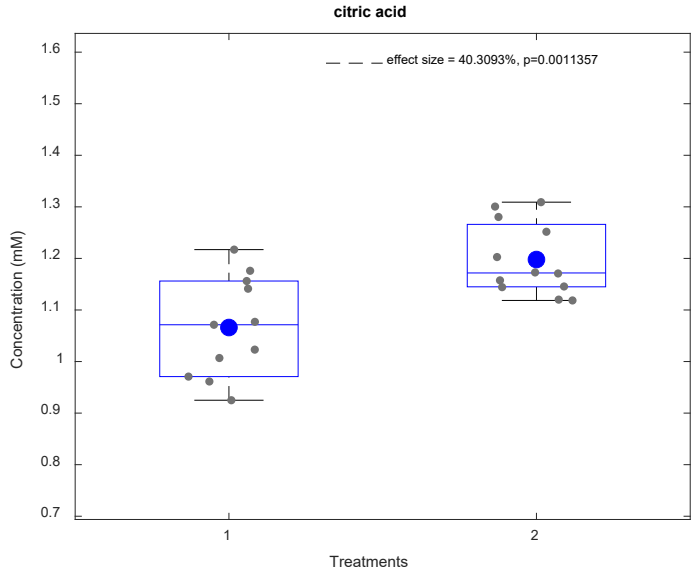
Discriminant metabolites

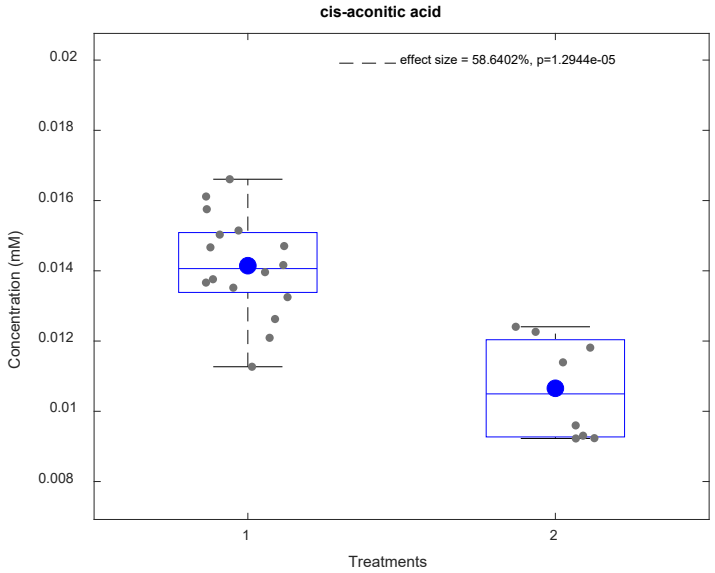


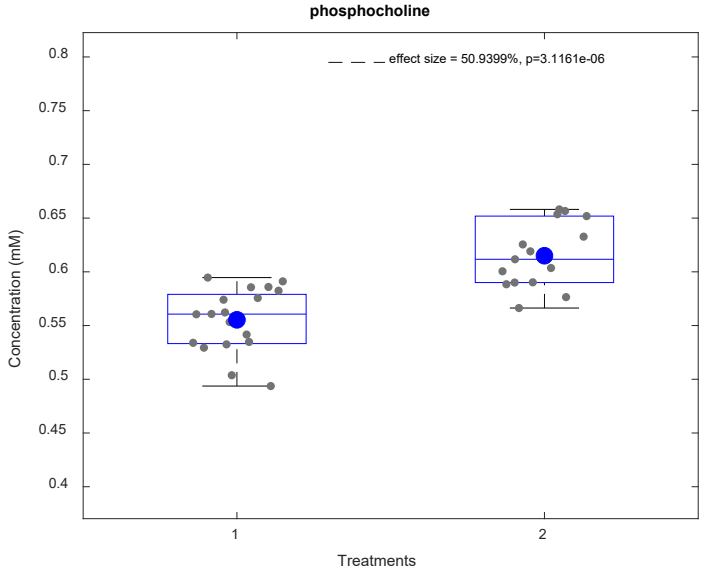


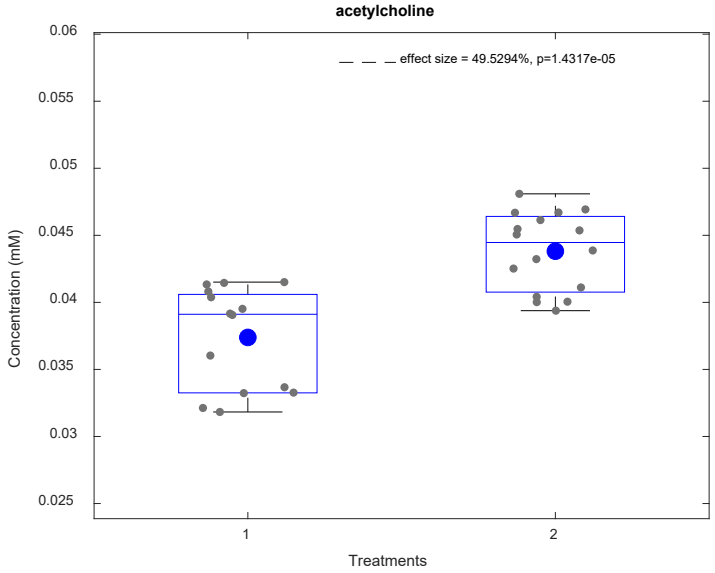


Discriminant metabolites

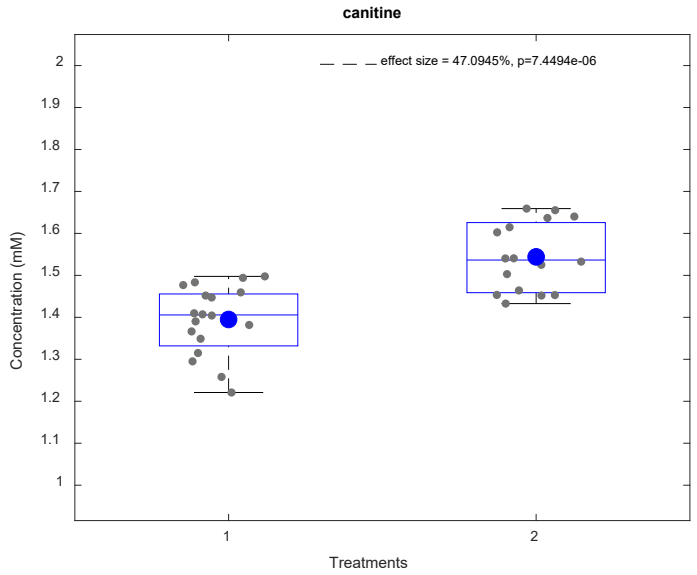


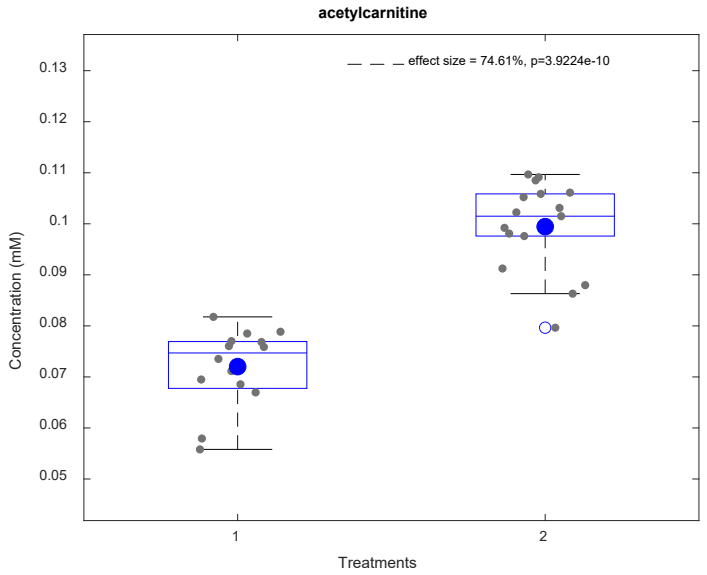


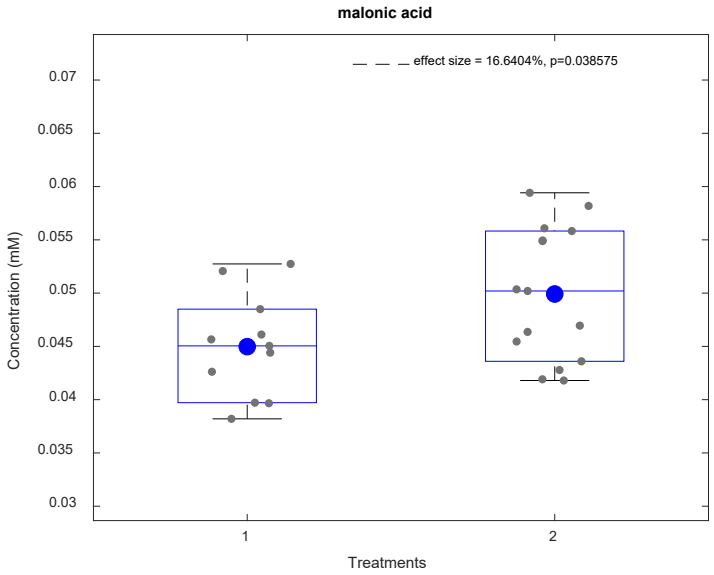


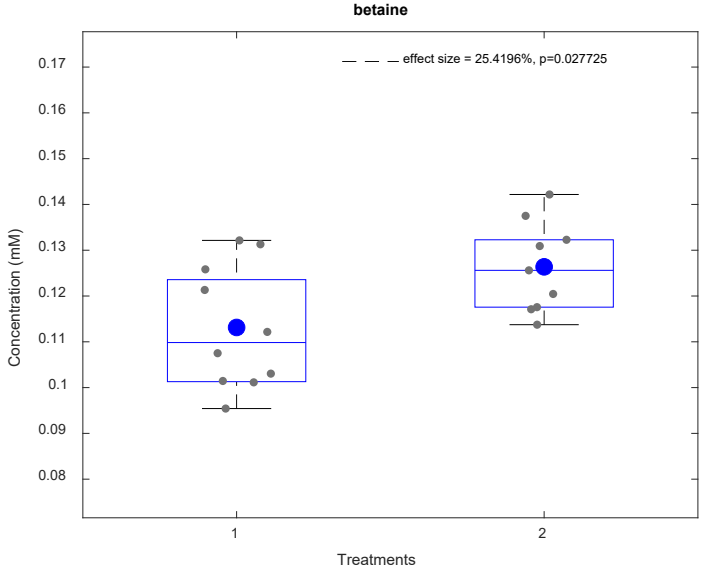


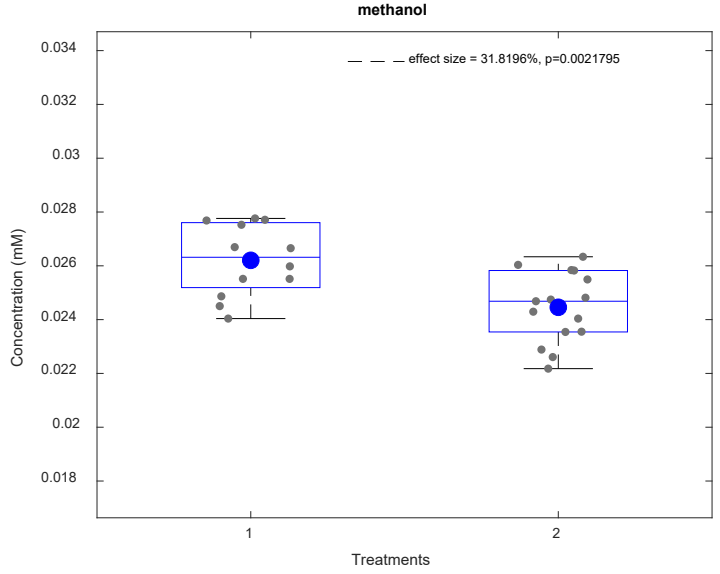
Discriminant metabolites

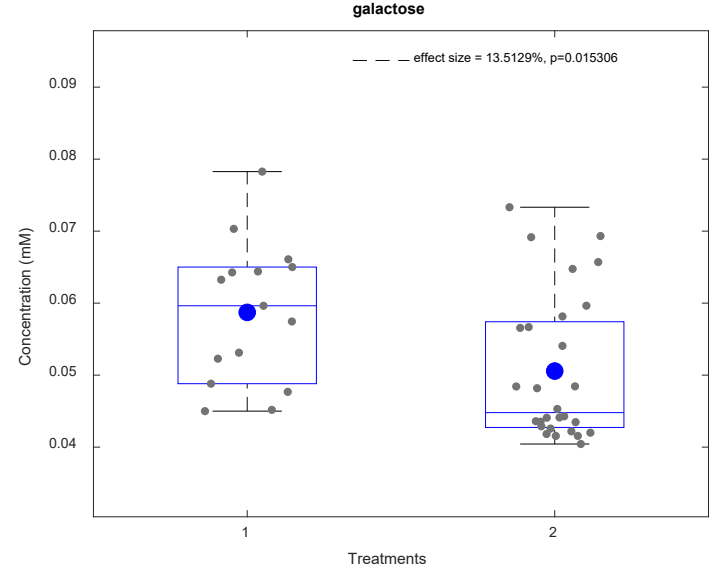




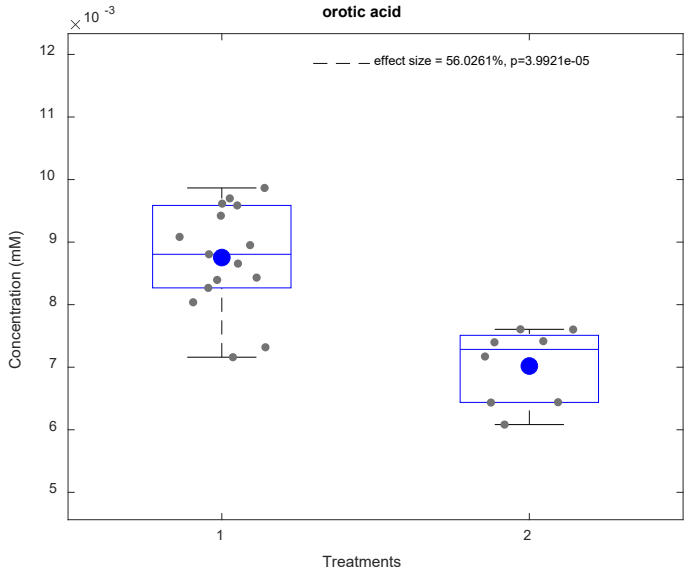


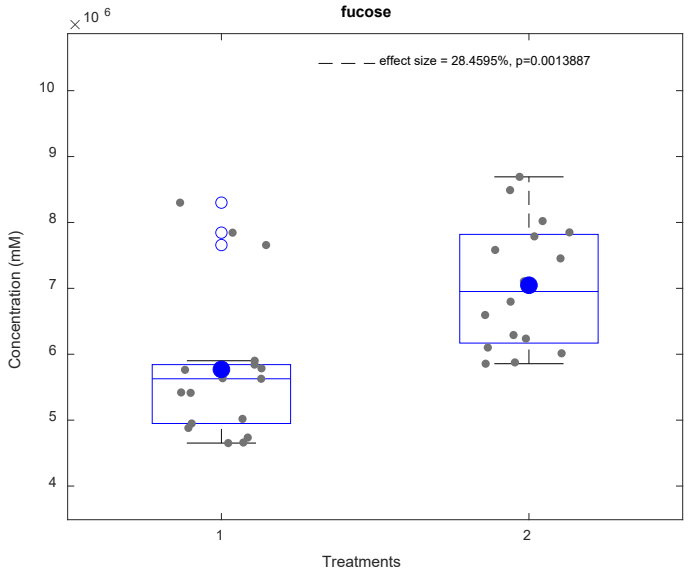


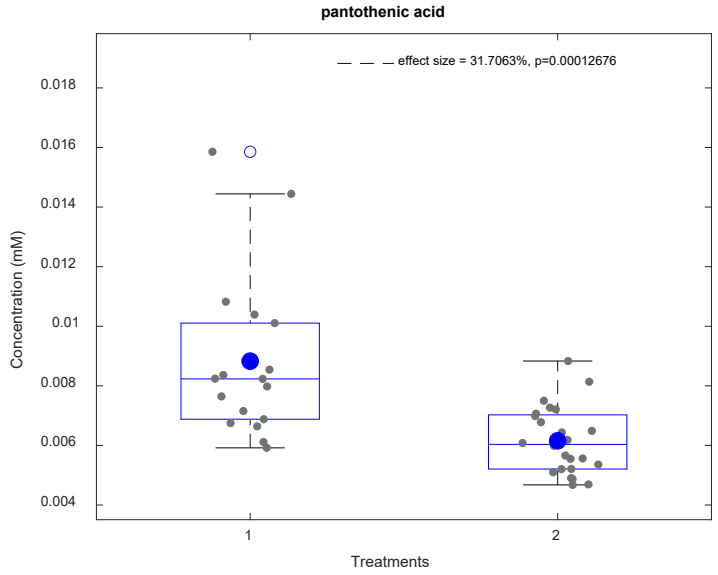




Discriminant metabolites







Conclusions on colostrum NMR

- 2021: dominant effect of energy metabolism, apoptotic mamary epithelial cells and feeding intake
- 2022: dominant effect of microbial activity
- 2023: dominant effect of energy metabolism and feeding

Urine NMR metabolomics

Aim

- To characterize the molecular composition of cow urine.
- To identify compounds correlated to urine pH and bone degradation

Samples (764 in total)

- 2021
- 2022
- 2023

Sample preparation

Thaw at room T

720 µl urine + 80 µl 7.4 mM phosphate buffer
(1 mg/ml TSP, 0.13 mg/ml NaN_3)

Centrifuge
(1.5K g, 30', 4°C)

Tranfer 700 µl
in NMR tube

Measure

Urine ^{1}H NMR spectrum

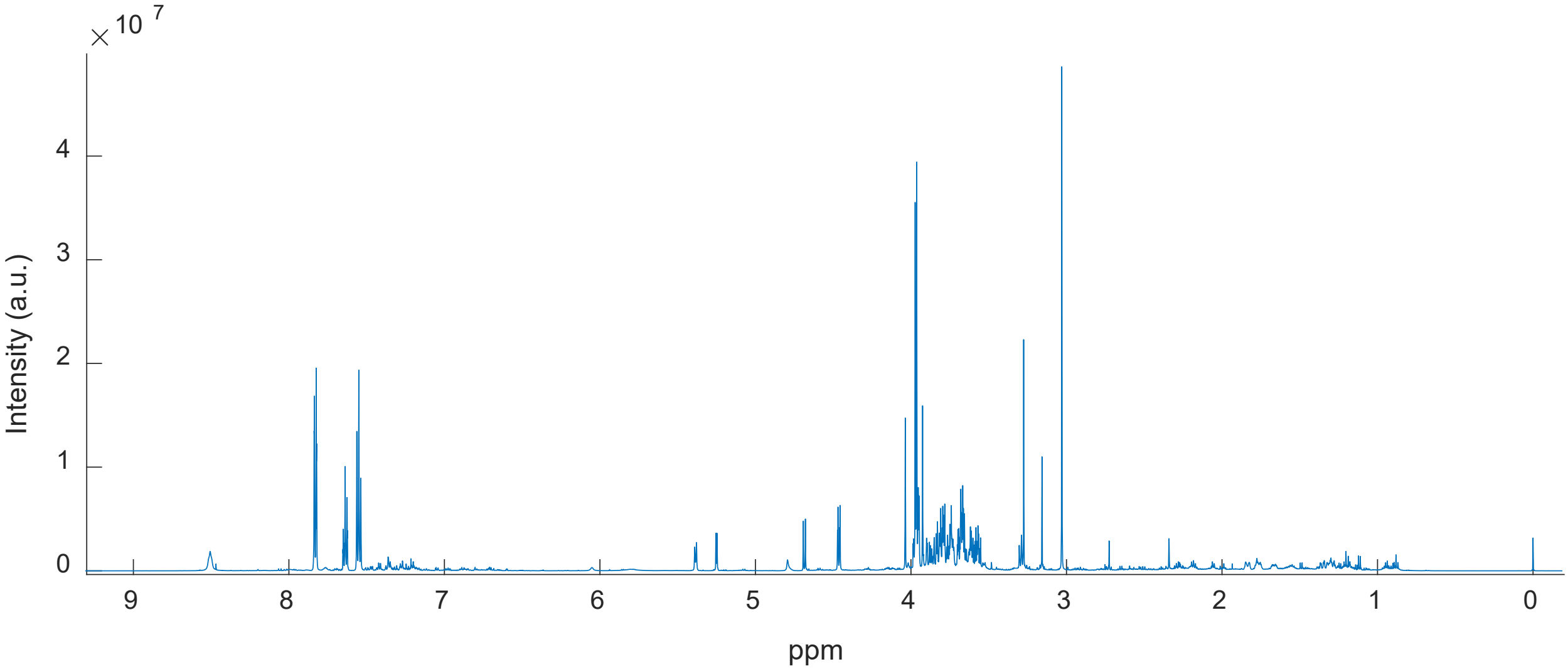


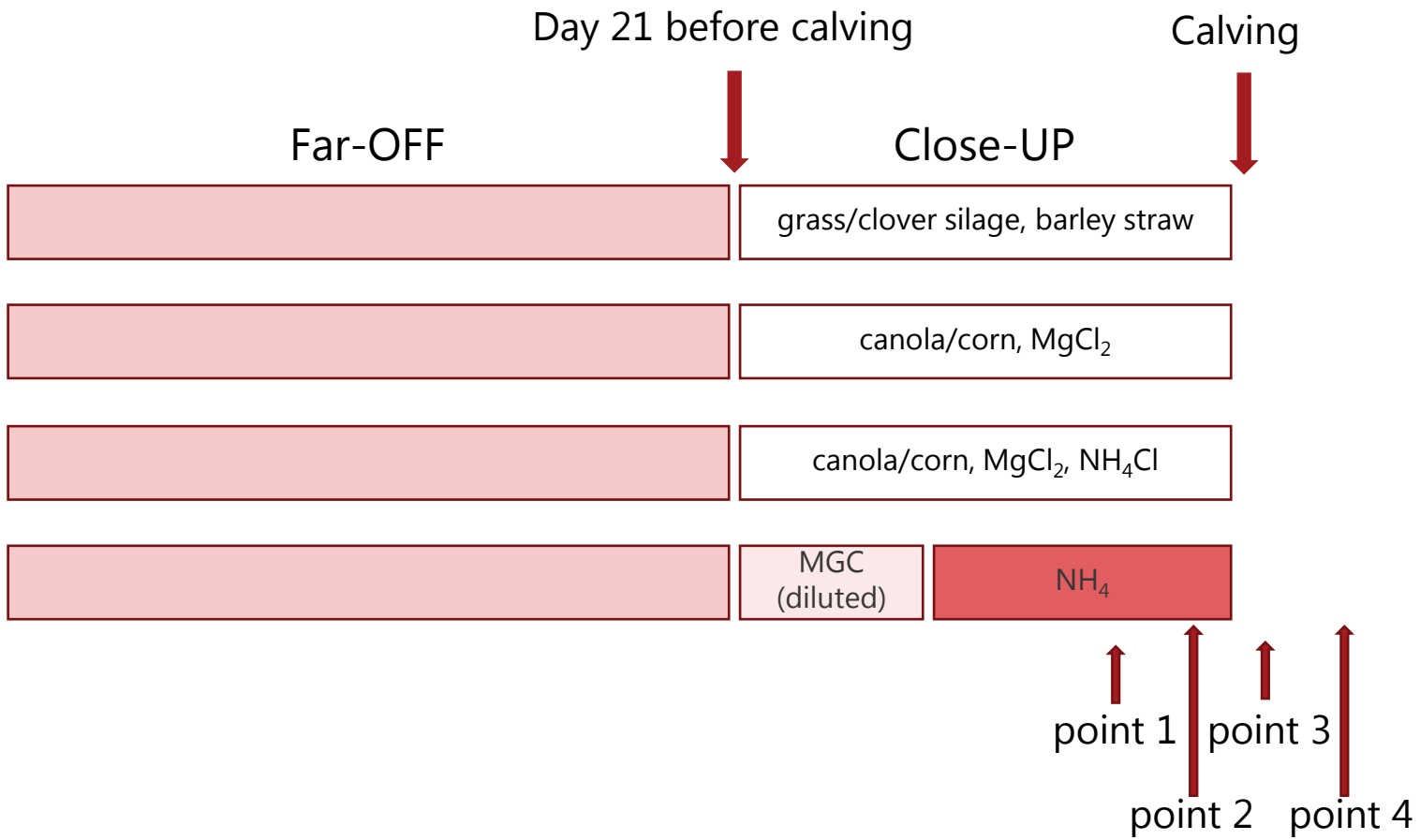
Table of identified metabolites (48)

2-hydroxybutyric acid	dimethylamine	p-cresor sulfate
2-hydroxyvaleric acid	ethanolamine	phenylacetylglutamine
3-hydroxybutyric acid	formate	phenylacetylglycine
3-methyl-2-oxovalerate	fucose	propylene glycol
acetate	fumarate	puryvic acid
acetoin	gluconic acid	riboflavin
acetone	hippurate	ribose
alanine	histidine	sebacid acid
allantoin	homovalinillic acid	sucrose
aspartic acid	lactate	taurine
betaine	lactose	threonine
carnosine	lactose	trimethylamine N-oxide
cellobiose	lysine	tyrosine
creatine	methylsuccinic acid	uracil
creatinine	niacinamide	urea
cytidine	pantothenic acid	xanthosine

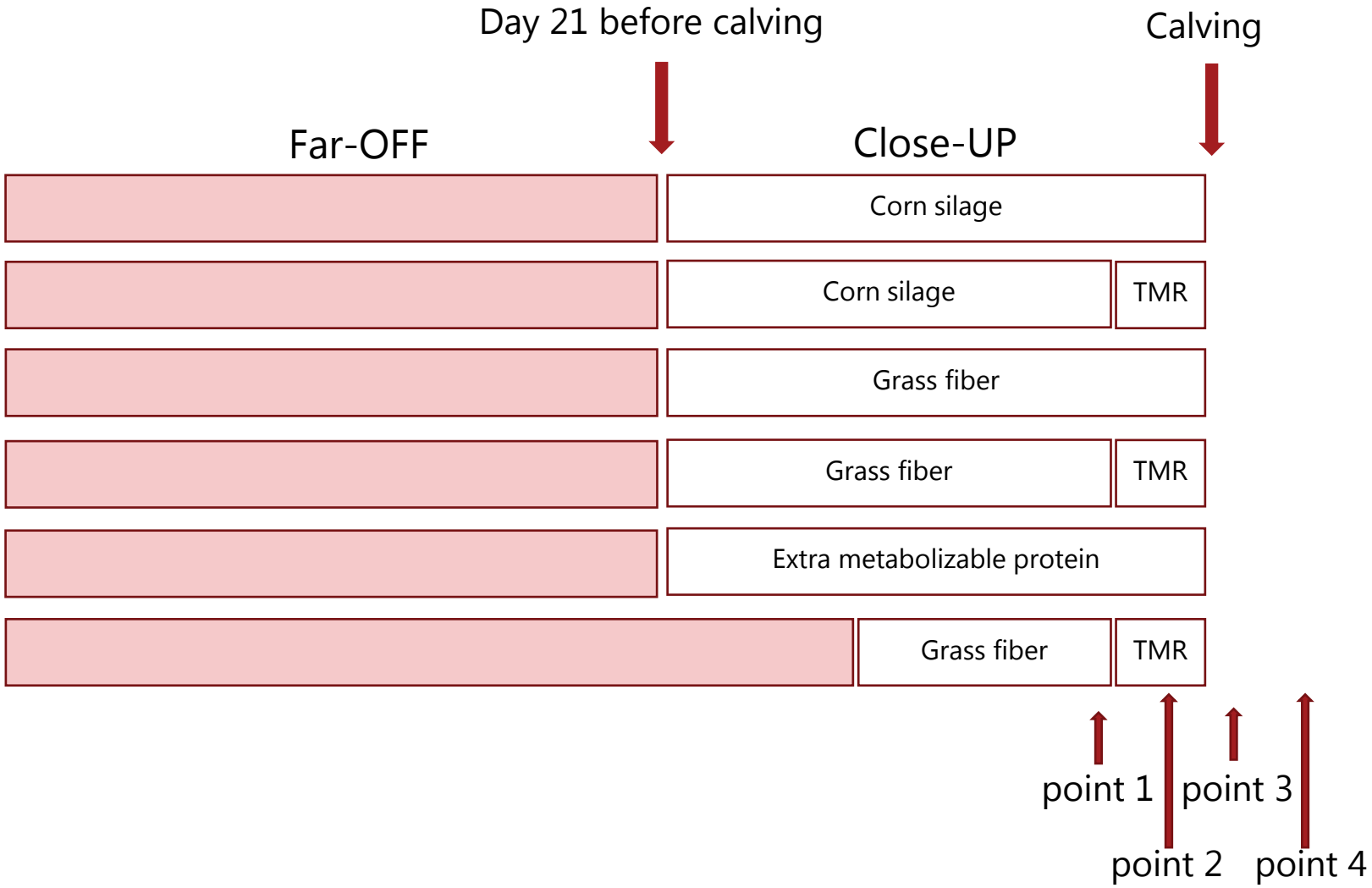
Target metabolites for identification

2-Hydroxyphenylacetate	**Desaminotyrosine**	N6-Acetyllysine
2-Hydroxyvalerate	Ferulate	**N-Acetylserotonin**
3,4-Dihydroxymandelate	Fructose	N-Acetyltyrosine
3-Hydroxy-3-methylglutarate	Galactarate	**N-Methylhydantoin**
3-Hydroxyisovalerate	**Gentisate**	**N-Nitrosodimethylamine**
3-Hydroxymandelate	**Glucose-6-phosphate**	O-Acetylcholine
3-Hydroxyphenylacetate	**Glucuronate**	Oxypurinol
3-Indoxylsulfate	Glycolate	Pyridoxine
3-Methylglutarate	**Glycylproline**	Salicylate
3-Methylxanthine	**Guanidoacetate**	Salicylurate
4-Pyridoxate	Histamine	Serotonin
5-Hydroxyindole-3-acetate	Indole-3-acetate	Thymidine
Acetylsalicylate	Indole-3-lactate	Thymol
Anserine	**Isocitrate**	trans-Aconitate
Arabinose	Maltose	Trimethylamine
Caffeine	Mandelate	Tryptophan
cis-Aconitate	Melatonin	**Vanillate**
Citraconate	**N,N-Dimethylglycine**	**Xanthurenate**

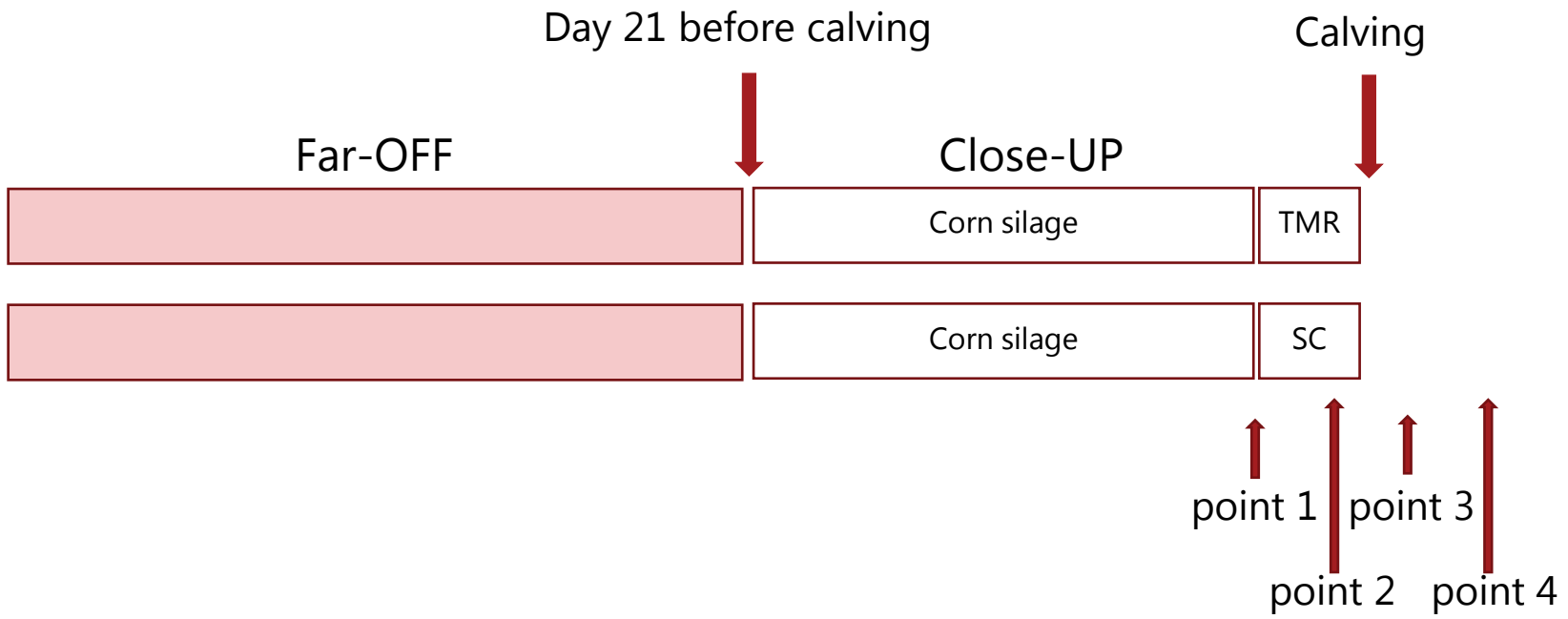
Urine samples design – 2021 (75 samples)



Urine samples design – 2022 (578 samples)



Urine samples design – 2023 (111 samples)



SC: special concentrate

In progress

- Finalize identification and statistical analysis for urine NMR data