European Digital UniverCity

EDUC-SHARE

WP2 RESEARCH INFRASTRUCTURES

Task 2.3 Unlocking the collaborative potential of EDUC Research Infrastructures

Advancing healthcare using the Research Infrastructure CeSAR of UniCa: Nutrition during the first two years of life

Cagliari, 23th and 24th November 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101017526

www.educalliance.eu

LIPIDOMICS AND ITS APPLICATIONS IN THE CLINIC AND NUTRITION

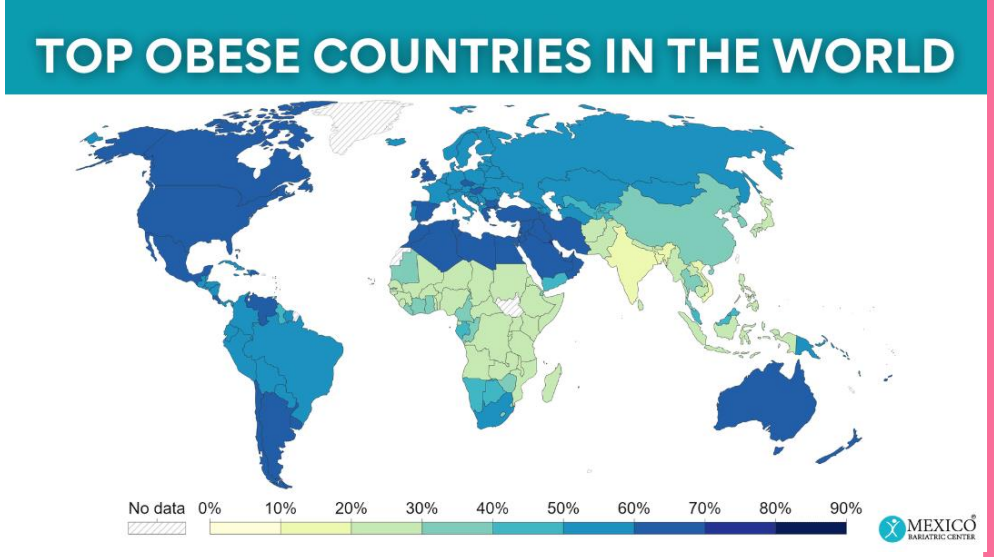
Prof. Pierluigi Caboni

Food research Lab

Department of environmental and life science

University of Cagliari

caboni@unica.it



Scientific context

- Diet-related diseases ↑
- Prevent these pathologies rather than cure them
- Gut = interface between food and human body. Digestion releases food components that can have a beneficial or a deleterious effect on human health
- the mechanisms of food disintegration in the gastrointestinal tract remain unclear and the digestive process has been considered as a black box so far
- by increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health

Application:

Comparative Lipidomics Exploration: Unraveling Common Characteristics in Sheep and Goat Infant Formulas after In Vitro Dynamic Digestion

- In the case of a newborn, milk is recognized as a comprehensive nourishment capable of meeting its physiological and energy needs throughout the developmental process.
  - Breast milk considered as a gold standard

Application:

Comparative Lipidomics Exploration: Unraveling Common Characteristics in Sheep and Goat Infant Formulas after In Vitro Dynamic Digestion

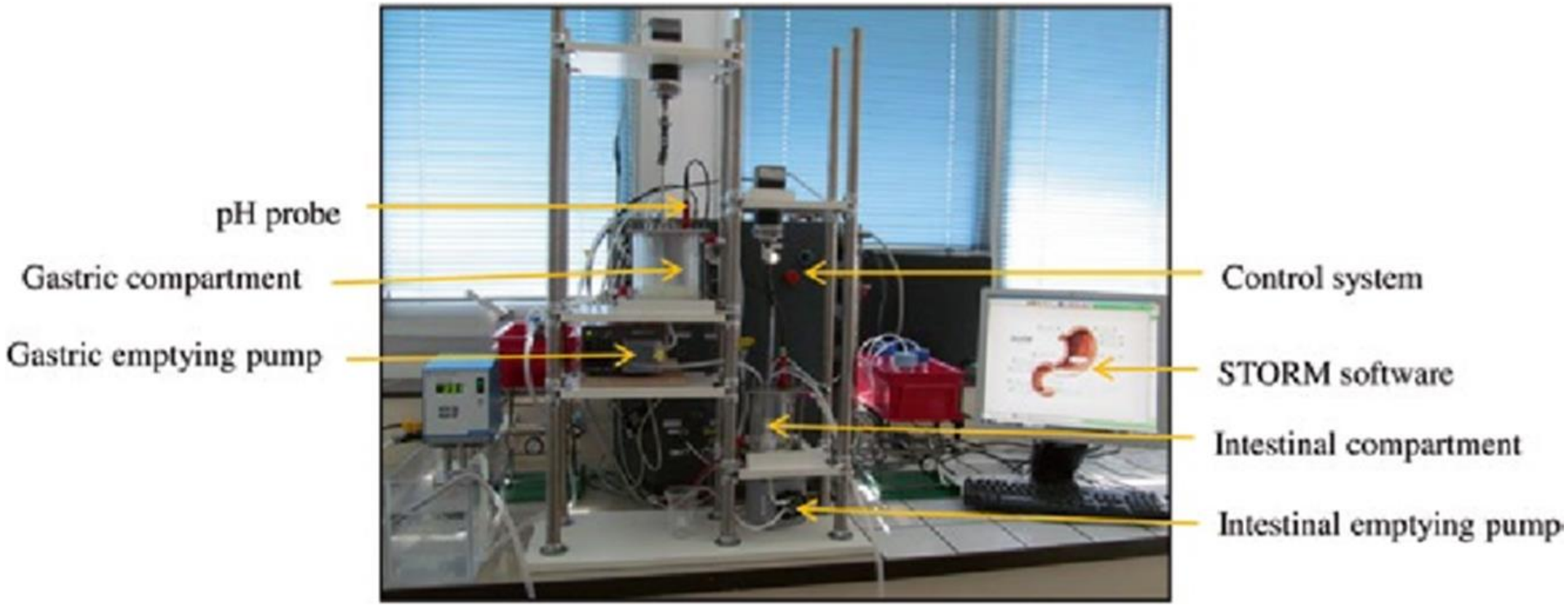
- For most of the commercial infant formulas (IFs), only the protein fraction originates from cow's (or goat) milk whereas lipids mainly correspond to a blend of vegetable oils (canola oil, safflower oil,)
  - **Sheep milk is not considered as a source of protein**
  - **Sardinia's ship milk production 300 M liters/year!**

The in vitro dynamic digestion system (DIDGI)

- used to mimic the stomach and small intestine activity after food consumption
- validated systems replicate food disintegration
- allow data collection for the simulation of kinetics of proteolysis and lipolysis.
- validated against in vivo methods to prove their physiological relevance

The in vitro dynamic digestion system (DIDGI)

Collaboration with Didier Dupont at STLO, INRAE, Institut Agro, 35042, Rennes, France



The in vitro dynamic digestion system (DIDGI)

- used to mimic the stomach and small intestine activity after food consumption.
- validated systems replicate food disintegration
- Allow data collection for the simulation of kinetics of proteolysis and lipolysis.
- validated against in vivo methods to prove their physiological relevance

The in vitro dynamic digestion system (DIDGI)

- system validated against in vivo methods to prove their physiological relevance.
- in the static in vitro digestion models, pH and enzyme concentration remain constant,
- dynamic in vitro model exhibits variations in pH and enzyme concentrations are dependent on the progression of time.
- hydrolysis of macronutrients are a function of time, pH, substrate, and enzyme concentrations.

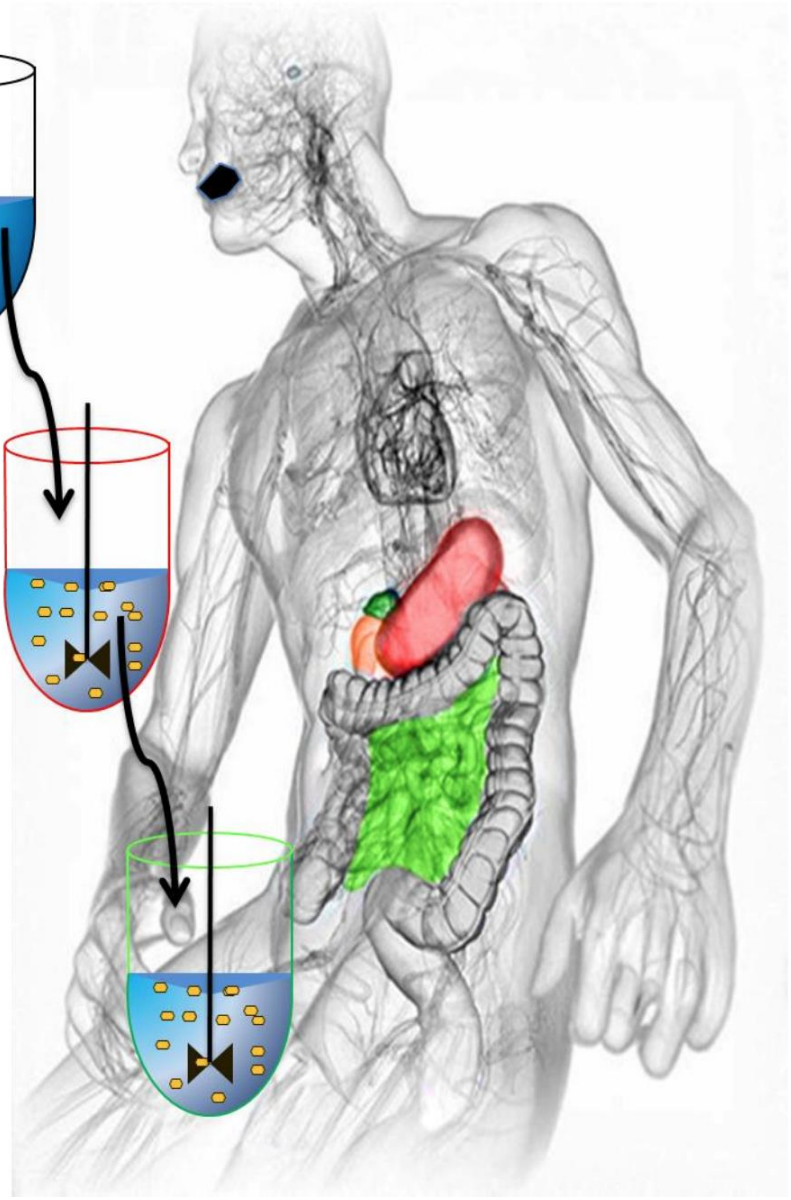
In vitro gastro-intestinal digestion
Consensus INFOGEST protocol

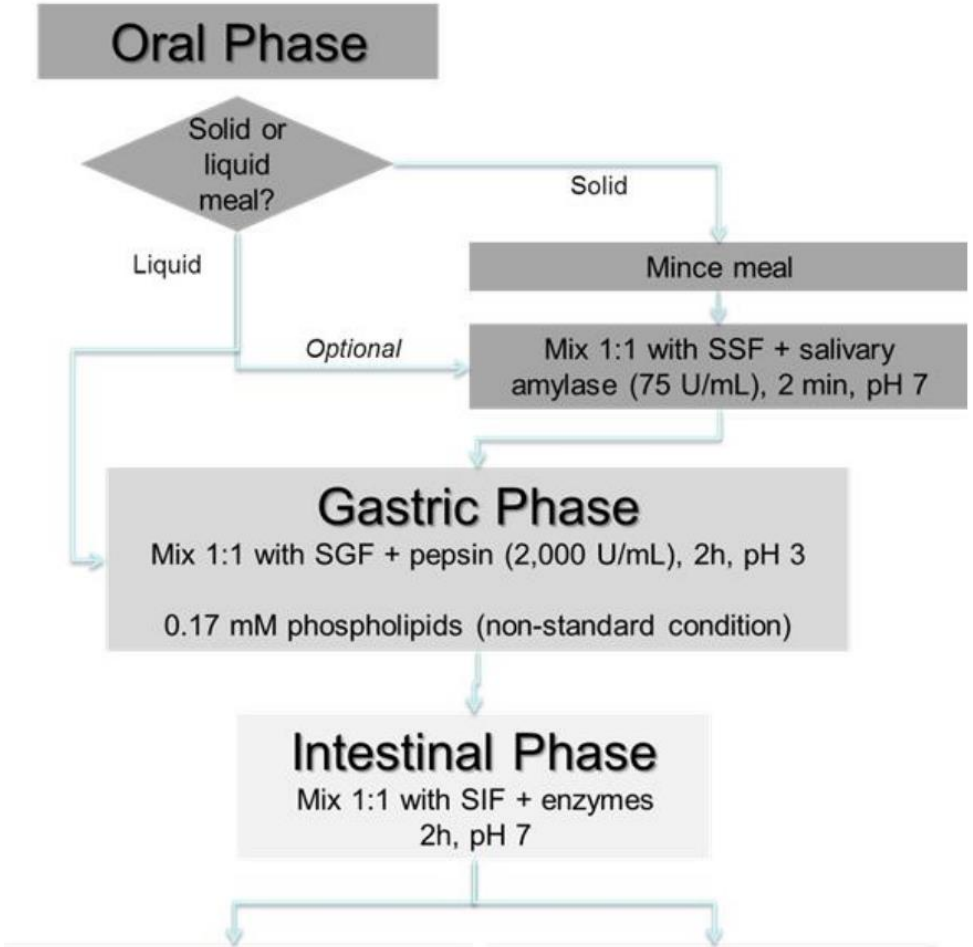
Oral phase
Mix 1:1 with Simulated Salivary Fluid (SSF)
salivary amylase (75 U/mL)
2 min, pH 7

Gastric Phase
Mix 1:1 with Simulated Gastric Fluid (SGF)
Pepsin (2000 U/mL)
2h, pH 3

Intestinal Phase
Mix 1:1 with Simulated Intestinal Fluid (SIF)
Enzymes
- Pancreatin (based on trypsin 100 U/mL) or
- Pure enzymes

Bile (10mM)
2h, pH 7





Minekus et al. 2014
Food Funct. 5, 1113-24
46 citations
Hot paper (0.1%)

The gastric phase

Ratio oral content / Simulated gastric fluid (SGF) : **50/50 w/v**
Porcine pepsin: **2000 U/mL**
Time of gastric digestion: **2 hours**
pH of the reaction: **3**

INFOGEST

Why 2 hours?
Duration highly depends on the type of food/meal

* Gastric emptying of a western type solid meal: 3-4h, of a liquid 0.5-1h
* Addition of nutrients to a liquid meal increases the transit time
* Strong inter and intra-individual variability

A time of 2h for gastric digestion represents the half emptying of a moderately nutritious and semi-solid meal

Why pH 3?
Fasted pH commonly found is around or below 2
pH increases to 5 and above because of the buffering capacity of the food/meal

pH 3 represents the mean value for a general meal exhibiting a gastric emptying half-time of 2 h

The intestinal phase

- ☞ Ratio Food (gastric content) / Simulated duodenal fluid (SDF): **50/50 w/v**
- ☞ Time of duodenal digestion: **2 hours**
- ☞ pH of the solution: **7**

INFOGEST

20 mL gastric content
+ 3.0 µL of **CaCl2** (H2O)2 (588 g/L, w/v)
+ Bile: (final concentration in total fluid 10 mM). There are two options for bile for the duodenal stage, which is to use either:

Bile extract (e.g. B8631-100G from Sigma) or
Fresh porcine bile (available from several InfoGest members including IFR (160 mM stock). The SDF the concentration is made up to 20mM.

+ fill up to a final volume of 40 mL with SDF to reach the same volume as the gastric digesta (20mL).

At this point there are two options in how to proceed:

1. Use pancreatin: sufficient pancreatin to provide 100 U/ml of trypsin (TAME Units). The proteolytic, lipolytic and amylolytic activity should be determined

2. Use individual enzymes

Simulated digestion fluids

			SSF		SGF		SIF	
			pH 7		pH 3		pH 7	
Constituent	Stock conc.		Vol. of stock	Conc. in SSF	Vol. of stock	Conc. in SGF	Vol. of stock	Conc. in SIF
	$g\ L^{-1}$	$mol\ L^{-1}$	mL	$mmol\ L^{-1}$	mL	$mmol\ L^{-1}$	mL	$mmol\ L^{-1}$
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH_2PO_4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
$NaHCO_3$	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	—	—	11.8	47.2	9.6	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.5	0.15	0.4	0.1	1.1	0.33
$(NH_4)_2CO_3$	48	0.5	0.06	0.06	0.5	0.5	—	—
For pH adjustment								
	$mol\ L^{-1}$		mL	$mmol\ L^{-1}$	mL	$mmol\ L^{-1}$	mL	$mmol\ L^{-1}$
NaOH	1		—	—	—	—	—	—
HCl	6		0.09	1.1	1.3	15.6	0.7	8.4
$CaCl_2(H_2O)_2$ is not added to the simulated digestion fluids, see details in legend								
	$g\ L^{-1}$	$mol\ L^{-1}$		$mmol\ L^{-1}$		$mmol\ L^{-1}$		$mmol\ L^{-1}$
$CaCl_2(H_2O)_2$	44.1	0.3		1.5 (0.75*)		0.15 (0.075*)		0.6 (0.3*)

[a] * in brackets is the corresponding Ca^{2+} concentration in the final digestion mixture.

applied sciences

MDPI

Article

Ion Mobility–Mass Spectrometry Approach for the Comparison of Sheep and Goat Milk Lipidomes

Cristina Manis [1], Paola Scano [1], Viviana Garau [2], Margherita Addis [3], Ignazio Ibba [4] and Pierluigi Caboni [1,*]

[1] Department of Life and Environmental Sciences, University of Cagliari, 09042 Cagliari, Italy
[2] Scuola di Specializzazione in Farmacia Ospedaliera, Dipartimento di Chimica e Farmacia, Università di Sassari, Via Muroni 23a, 07100 Sassari, Italy
[3] Agris Sardegna, Servizio Ricerca Prodotti di Origine Animale, Loc. Bonassai, 07040 Olmedo, Italy
[4] Associazione Regionale Allevatori, Laboratorio Analisi del Latte, Loc. Palloni, Nuraxinieddu, 09170 Oristano, Italy
* Correspondence: caboni@unica.it; Tel.: +39-0706758617

Sheep Milk Infant Formula
婴儿配方羊奶粉

Ingredients:

Whole Sheep Milk, Sheep Demineralized Whey Powder, Lactose, Vegetable Oils (Sunflower Oil, Canola Oil, Coconut Oil), Sheep Concentrated Whey Protein Powder, Galactooligosaccharide, Minerals (Potassium Citrate, Tricalcium Phosphate, Sodium Chloride, Potassium Chloride, Magnesium Sulphate, Calcium Carbonate, Ferrous Sulphate, Zinc Sulphate, Copper Sulphate, Sodium Selenite, Manganese Sulphate, Potassium Iodide), Fructooligosaccharide, Vitamins (Choline Chloride, Sodium L-Ascorbate, dl-α-Tocopheryl Acetate, Inositol, Vitamin A Acetate, Niacinamide, Cholecalciferol, Riboflavin, Calcium D-Pantothenate, Phytonadione, Thiamine Hydrochloride, Pyridoxine Hydrochloride, Cyanocobalamin, Folic Acid, D-Biotin), ARA Oil, DHA Oil, Taurine,Nucleotides (Cytidine 5' - Monophosphate, Adenosine 5' - Monophosphate, Inosine 5' - Monophosphate Sodium Salt, Guanosine 5' - Monophosphate Sodium Salt, Uridine 5' - Monophosphate Sodium Salt), L-Carnitine, Lutein.

配料:

全脂绵羊乳、脱盐乳清粉(来源:绵羊)、乳糖、植物油(葵花籽油、菜籽油、椰子油)、浓缩乳清蛋白粉(来源:绵羊)、低聚半乳糖、矿物质(柠檬酸钾、磷酸三钙、氯化钠、氯化钾、硫酸镁、碳酸钙、硫酸亚铁、硫酸锌、硫酸铜、亚硒酸钠、硫酸锰、碘化钾)、低聚果糖、维生素(氯化胆碱、L-抗坏血酸钠、dl-α-醋酸生育酚、肌醇、醋酸维生素A、烟酰胺、胆钙化醇、核黄素、D-泛酸钙、植物甲萘醌、盐酸硫胺素、盐酸吡哆醇、氰钴胺、叶酸、D-生物素)、花生四烯酸油脂、二十二碳六烯酸油脂、牛磷酸、核苷酸(5'单磷酸胞苷、5'单磷酸腺苷、5'-肌苷酸二钠、5'-鸟苷酸二钠、5'-尿苷酸二钠)、L-肉碱、叶黄素。

Storage:

Store can in a cool, dry place. Don't use if the seal is damaged. After opening, please keep it tightly closed after preparation, and use contents within 4 weeks. Verify manufacture date (yyyy/mm/dd), expiry date (yyyy/mm/dd) and batch number on the bottom of the tin. Please use the product before expiry date.

贮存条件:

请在阴凉干燥处贮存产品。如封口被损坏,请不要使用。开封后,每次使用完请将罐盖盖紧,并在4周内食用完。生产日期(年/月/日)、保质期至(年/月/日)及产品批号见罐底。请在保质期内使用本产品。

NUTRITION INFORMATION 营养成分表

Items	项目	Unit 单位	Per 100 kJ 每100千焦	Per 100g 每100克
Energy	**能量**	kJ 千焦	100	2082
Protein	**蛋白质**	g克	0.52	10.8
Whey Protein	乳清蛋白	g克	0.32	6.7
Fat	**脂肪**	g克	1.21	25.2
Linoleic Acid	亚油酸	g克	0.17	3.6
α-Linolenic Acid	α-亚麻酸	mg毫克	20.6	429
Carbohydrate	**碳水化合物**	g克	2.67	55.6
Vitamins	**维生素**			
Vitamin A	维生素A	μg RE微克视黄醇当量	22.6	471
Vitamin D	维生素D	μg微克	0.33	6.8
Vitamin E	维生素E	mgα-TE 毫克α-生育酚当量	0.42	8.8
Vitamin K_1	维生素K_1	μg微克	2.04	42.5
Vitamin B_1	维生素B_1	μg微克	19.7	410
Vitamin B_2	维生素B_2	μg微克	40.8	850
Vitamin B_6	维生素B_6	μg微克	17.3	360
Vitamin B_{12}	维生素B_{12}	μg微克	0.09	1.8
Nicotinic Acid	烟酸	μg微克	183	3800
Folic Acid	叶酸	μg微克	4.08	85
Pantothenic Acid	泛酸	μg微克	125	2600
Vitamin C	维生素C	mg毫克	3.60	75
Biotin	生物素	μg微克	0.76	15.8
Minerals	**矿物质**			
Sodium	钠	mg毫克	5.86	122
Potassium	钾	mg毫克	24.0	500
Copper	铜	μg微克	17.8	370
Magnesium	镁	mg毫克	1.54	32
Iron	铁	mg毫克	0.26	5.5
Zinc	锌	mg毫克	0.17	3.5
Manganese	锰	μg微克	2.93	61
Calcium	钙	mg毫克	16.8	350
Phosphorus	磷	mg毫克	10.5	219
Iodine	碘	μg微克	2.88	60
Chloride	氯	mg毫克	15.3	318
Selenium	硒	μg微克	0.63	13.2
Optional Items	**可选择性成分**			
Choline	胆碱	mg毫克	5.76	120
Inositol	肌醇	mg毫克	1.83	38
Taurine	牛磷酸	mg毫克	1.92	40
L-Carnitine	左旋肉碱	mg毫克	0.54	11.2
DHA	二十二碳六烯酸	%总脂肪酸	0.25	0.25
ARA	二十碳四烯酸	%总脂肪酸	0.38	0.38
Galactooligosaccharide	低聚半乳糖	g克	0.06	1.2
Fructooligosaccharide	低聚果糖	g克	0.06	1.2
Lutein	叶黄素	μg微克	3.12	65
Nucleotides	核苷酸	mg毫克	1.12	23.4

blue river 蓝河®

SHEEP MILK INFANT FORMULA (FOR 0-6 MONTHS)

婴儿配方羊奶粉
(0-6月龄，1段)

1

产品配方注册号:
国食注字YP20175071

净含量:**800g**

冲调方法:

1、冲调奶粉前,请将双手洗干净。
2、将洗净的奶瓶、奶嘴及瓶盖置入沸水中至少煮5分钟。
3、饮用水煮沸后,待凉至约45°C。参照建议喂哺表,将正确量的温开水倒入奶瓶中。
4、使用置于罐盖内的专用量匙,量取奶粉,并用洁净刀具沿匙口刮平,每30毫升温开水加入一平匙奶粉(约4.5克)。
5、摇动奶瓶,直至奶粉完全溶解为止。
6、喂哺前,先将奶液滴在手腕内侧测试奶温。

Preparation Method:

1. Always wash hands before preparing the formula.
2. Wash the bottle, teat and cap thoroughly, and boil the utensils for at least 5 minutes.
3. Boil fresh drinking water and cool it down to about 45°C. Add the correct quantity of warm boiled water into the bottle consulting the feeding table.
4. Fill the enclosed scoop with powder, and level off with a clean knife. Always add one level scoop of powder (about 4.5g) for each 30mL of warm boiled water.
5. Shake or stir until the powder is completely dissolved.
6. Test temperature on wrist before feeding.

SUGGESTED FEEDING TABLE 建议喂哺表

Age 年龄	Warm Boiled Water 温开水用量 (mL) (毫升)	Level Scoops 奶粉匙数	Feedings in 24 hours 每日喂哺次数
0-2周 (weeks)	60	2	9
2-4周 (weeks)	120	4	6
1-2个月 (months)	150	5	6
2-6个月 (months)	180	6	5

IMPORTANT NOTICE: BREAST MILK IS BEST FOR BABIES FROM 0 TO 6 MONTHS. IF THIS IS INSUFFICIENT OR YOU ARE UNABLE TO BREAST FEED THEN YOU CAN USE THIS PRODUCT AS A NOURISHING SUBSTITUTE BEFORE YOU DECIDE TO USE THIS PRODUCT, CONSULT YOUR DOCTOR OR HEALTH WORKER FOR ADVICE.

重要说明:对于0-6月的婴儿最理想的食品是母乳。在母乳不足或无母乳时可食用本产品。建议在使用本产品之前咨询专业医护人员。

WARNING: FOLLOW INSTRUCTIONS EXACTLY. PREPARE BOTTLES AND TEATS AS DIRECTED. DO NOT CHANGE PROPORTION OF POWDER EXCEPT ON MEDICAL ADVICE. INCORRECT PREPARATION CAN MAKE YOUR BABY VERY ILL.

- Only prepare one bottle each time, and feed immediately after preparation. Discard any unfinished feeds.

注意事项:请严格按照冲调方法冲调。请按说明清洁奶瓶和奶嘴。除非有专业医护人员建议,请勿调整冲调比例。不恰当的冲调比例可能会造成宝宝不适。

- 每次冲调一餐的用量,冲调后请立即喂哺。喂哺后剩余的奶液请弃掉。

9 421900 850631

更多信息
请关注官方微信公众号

MANUFACTURED AND PACKED IN NEW ZEALAND
新西兰原产原罐出品

产品类别:GB10765之乳基婴儿配方食品
原产国:新西兰
生产商:蓝河乳业有限合伙公司Blue River Dairy LP
生产商地址:新西兰因弗卡吉尔市尼斯街111号
111 Nith Street, Invercargill, New Zealand
生产商注册编号:595
中国总代理:蓝河营养品有限公司
地址:长沙市望城区仁和路7号
邮编:410200
咨询热线:400-966-1228
网址:www.blueriverdairy.com

Challenge

We employed a lipidomic approach to compare the hydrolysis profiles of lipids in 0-6 months infant formulas (IF) based on sheep and goat milk during dynamic in vitro digestion.

- By using a UHPLC-QTOF-MS platform, complex lipids were continuously detected and compared over time.
- The complete characterization of lipids was achieved through high resolution mass spectrometry experiments (mass fragmentation).
- Additionally, the lipid profile and the alteration of fatty acids as methyl esters (FAME) were measured for sheep and goat IFs and for the mixture of vegetable oils added to the formulation.

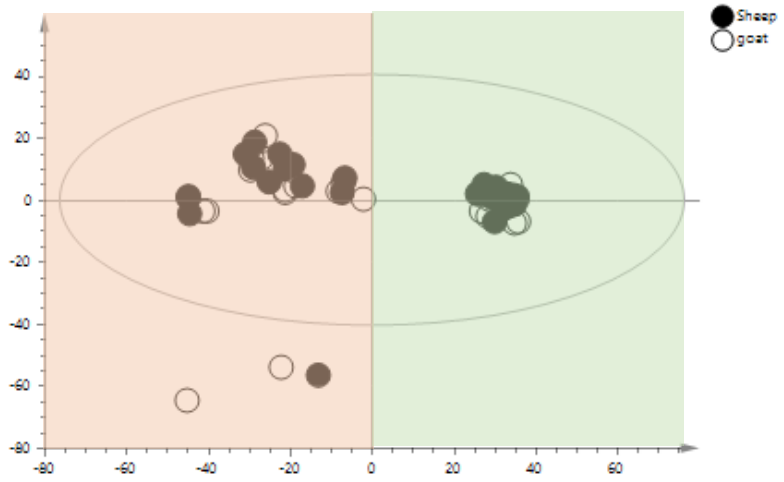
EDUC-SHARE
European Digital UniverCity

Percent gross composition of sheep and goat IFs 0-6 months.

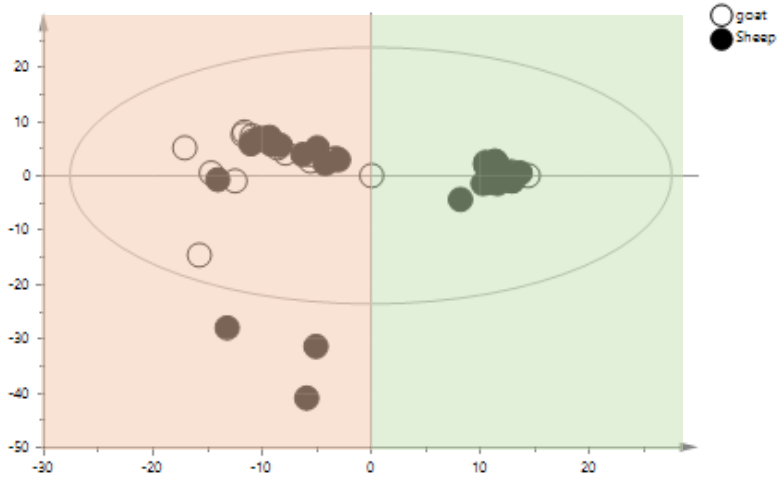
IF	Protein (%)	Whey protein (%)	Lipids (%)	Carbohydrates (%)	Net energy (KJ/100g)
Sheep milk	10.8	6.7	25.2	55.6	2082
Goat milk	10.8	6.7	26.0	54.4	2096

Principal component analysis (PCA) score plots

ESI+



ESI-

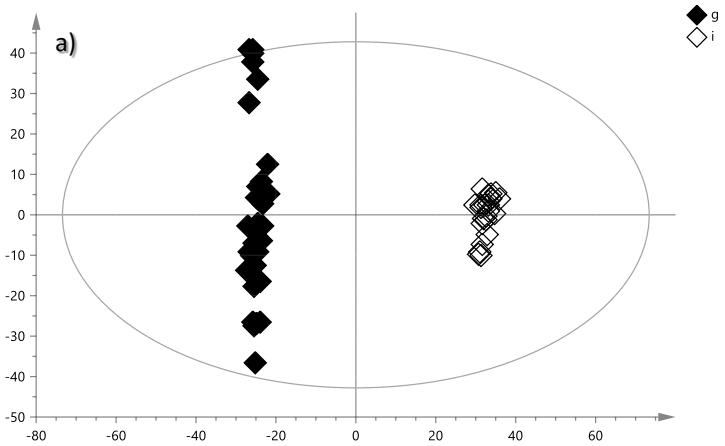


Stomach Gut

Unpublished data

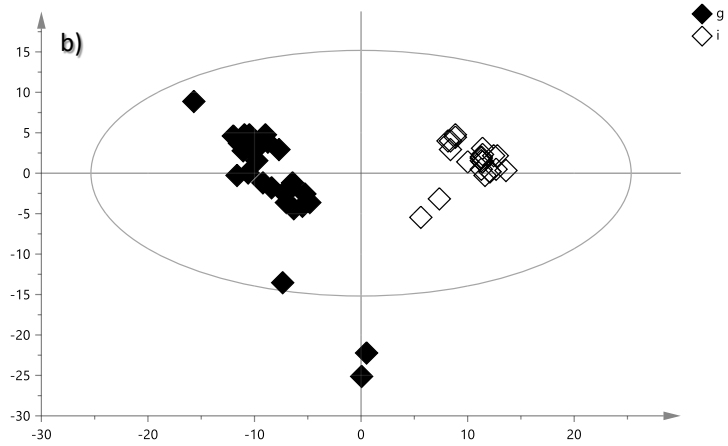
OPLS-DA score plots

ESI+



Stomach Gut

ESI-



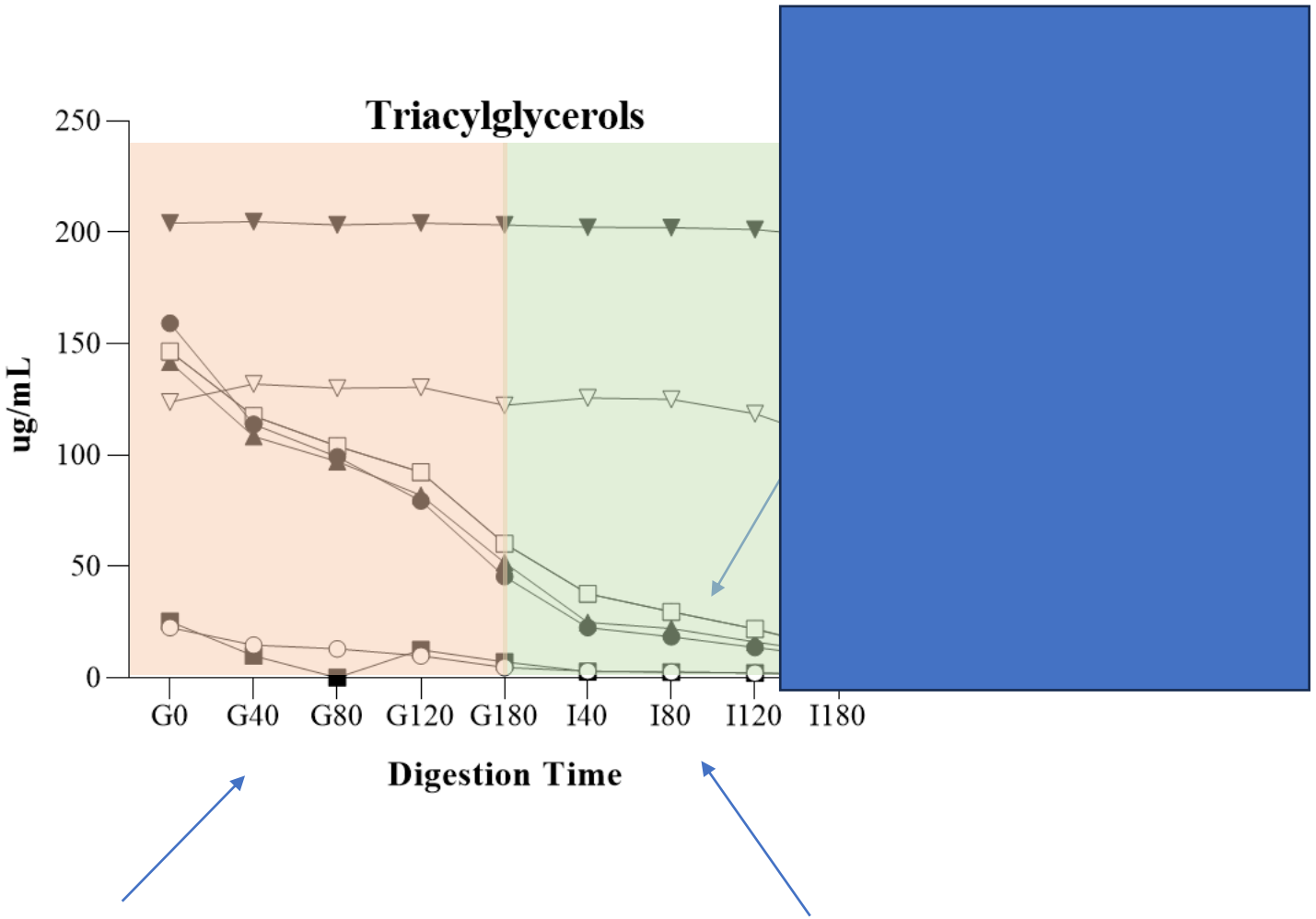
Stomach Gut

Unpublished data

Discriminant metabolites

Unpublished data

TG → DG +FA → 2MG + FA

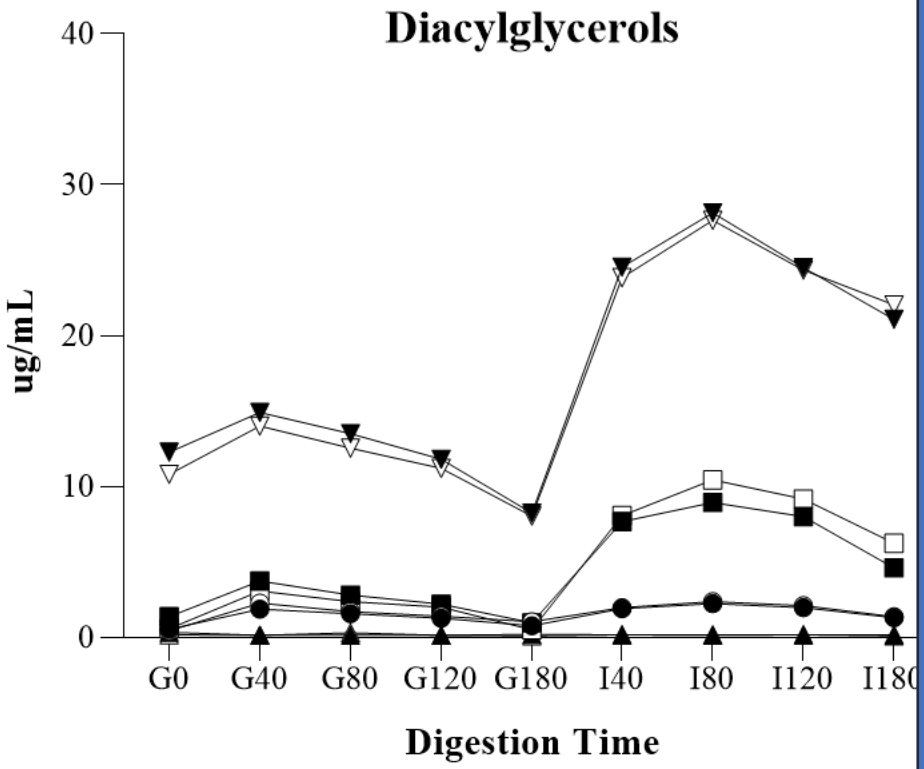


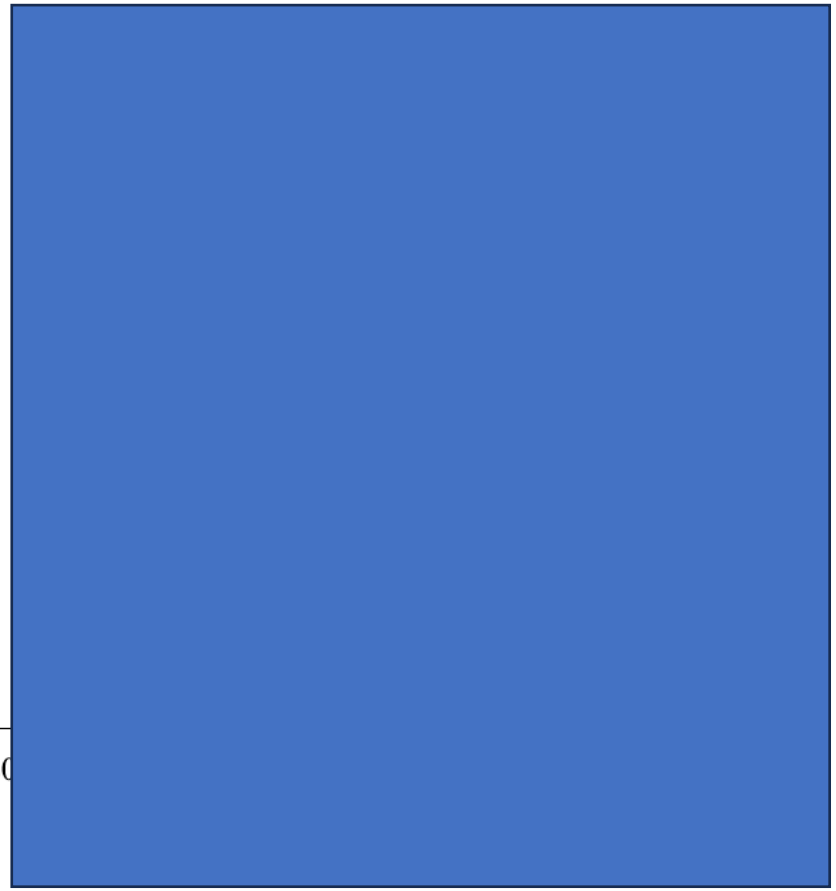
Gastric lipase (pH 3)

Intestinal lipase (pH 7)

Unpublished data

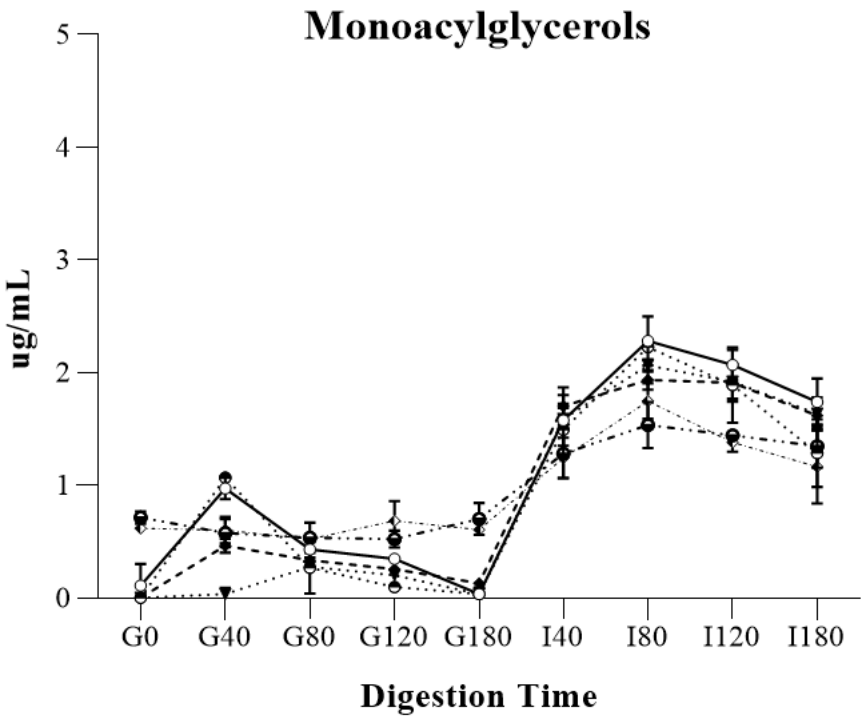
TG → DG → 2MG

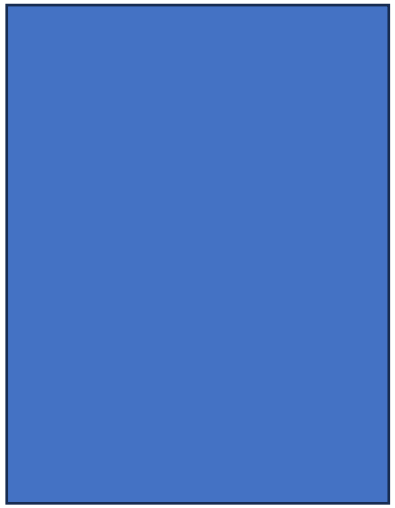




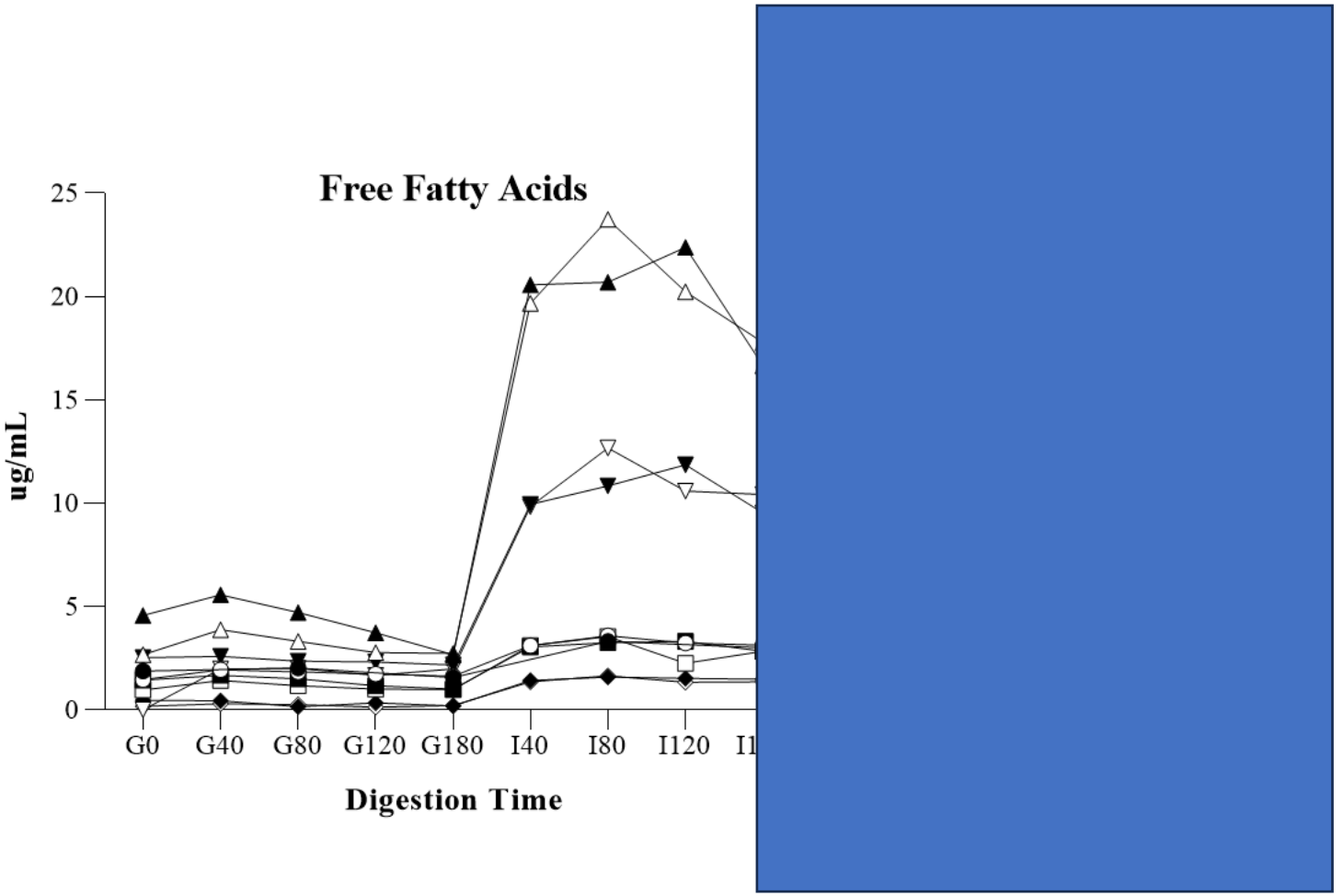
Unpublished data

TG → DG → 2MG

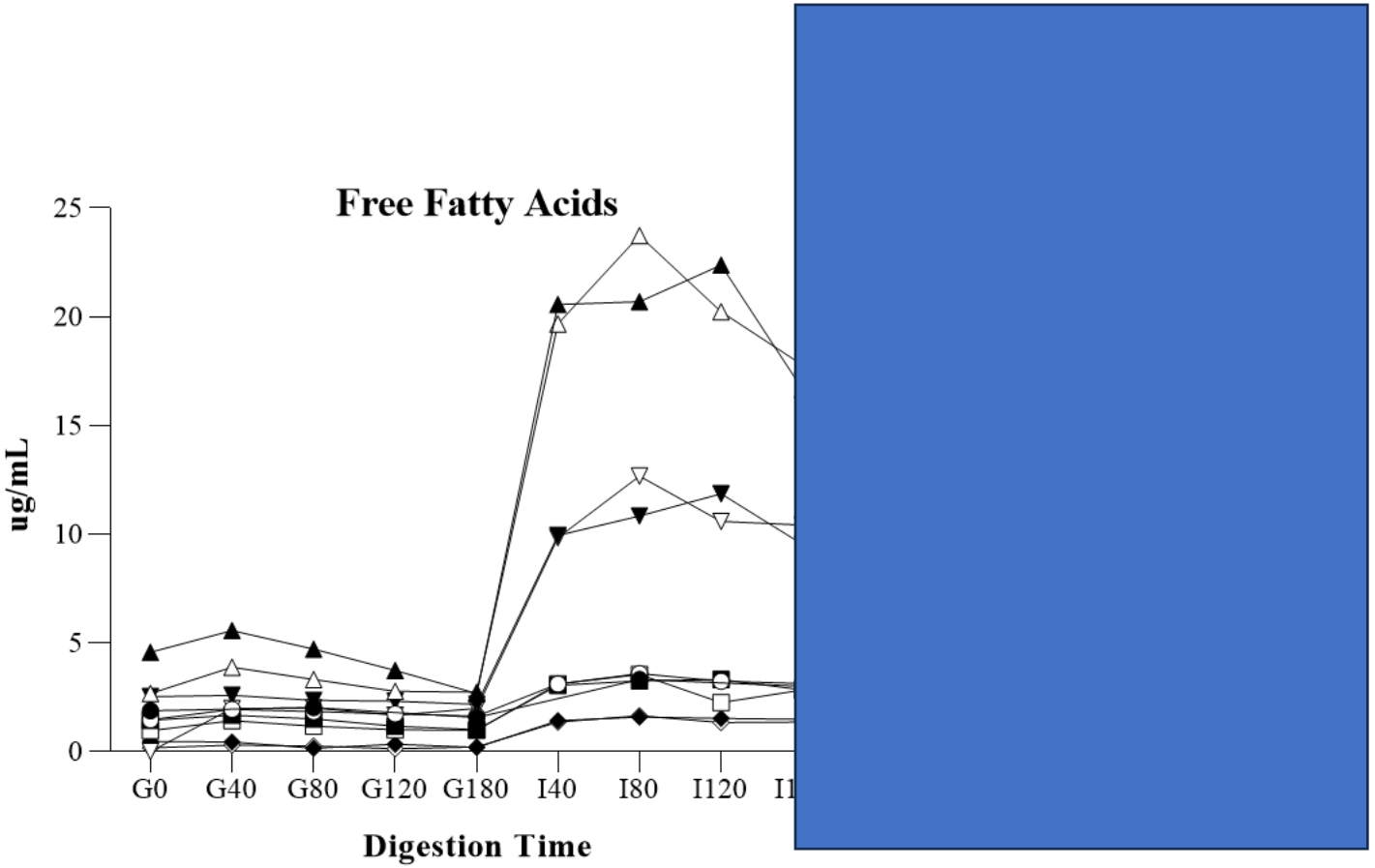




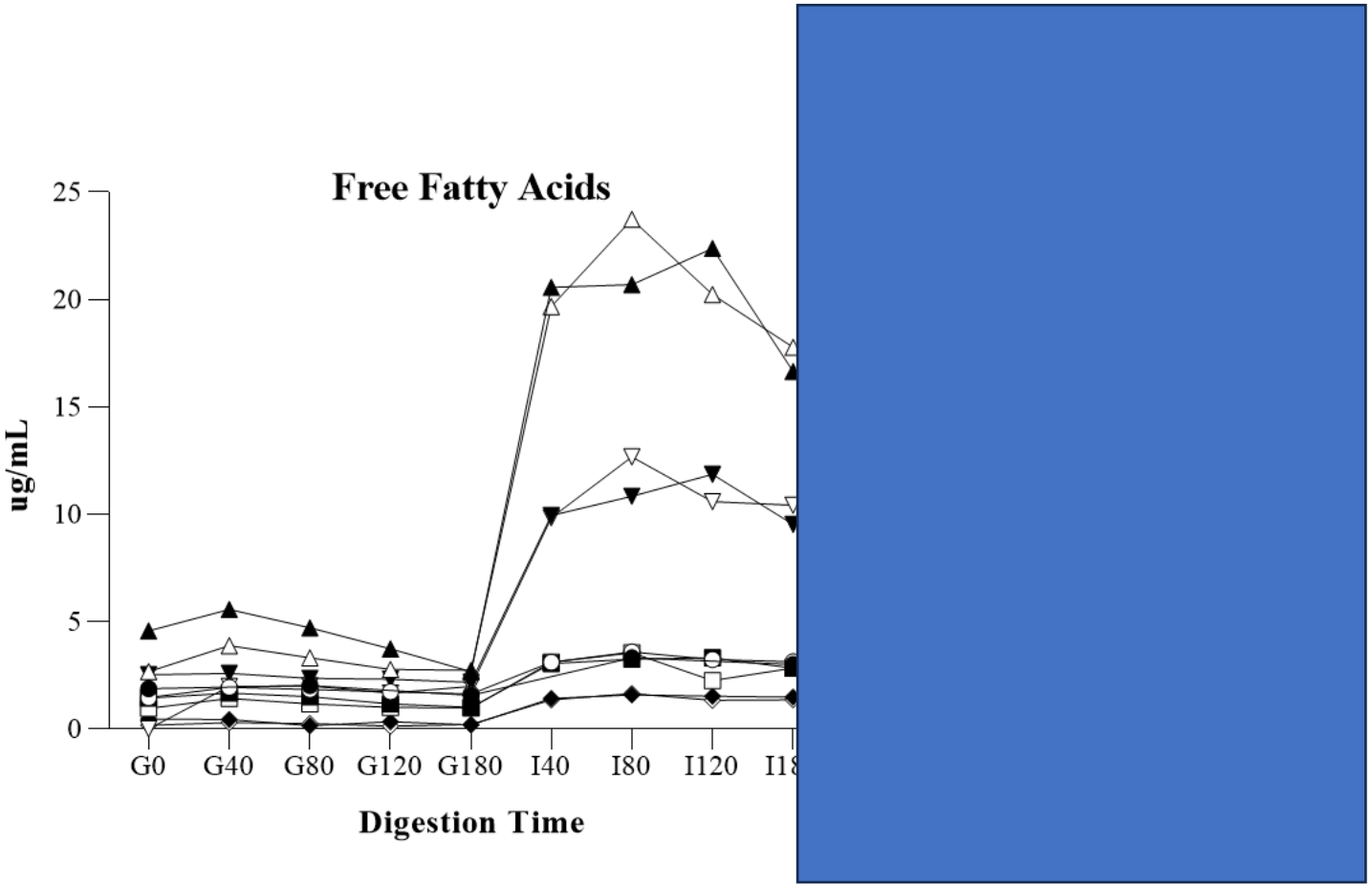
Unpublished data



Unpublished data

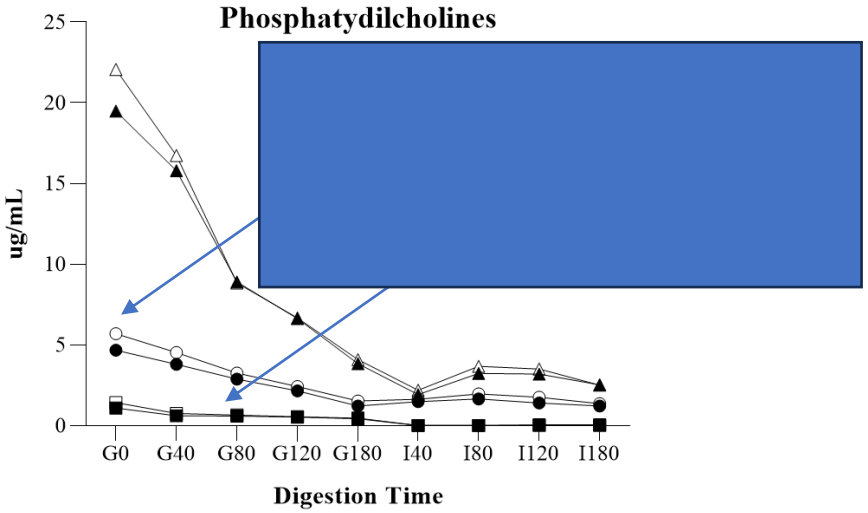


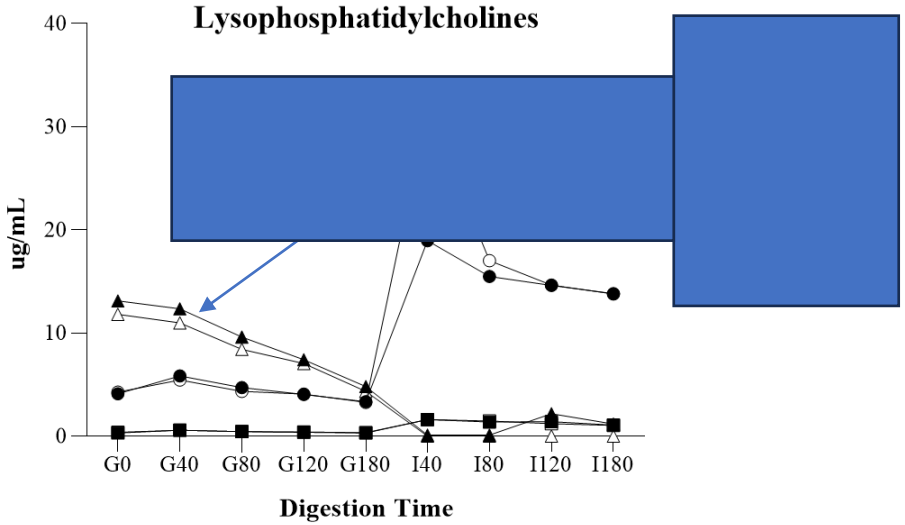
Unpublished data

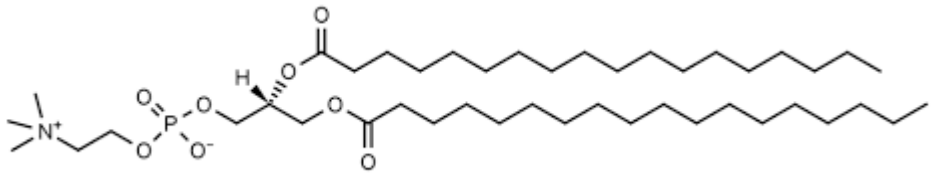


Unpublished data

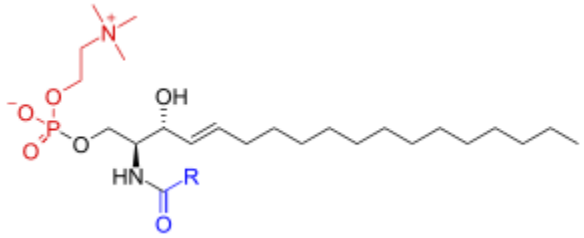
PC → LPC + FA

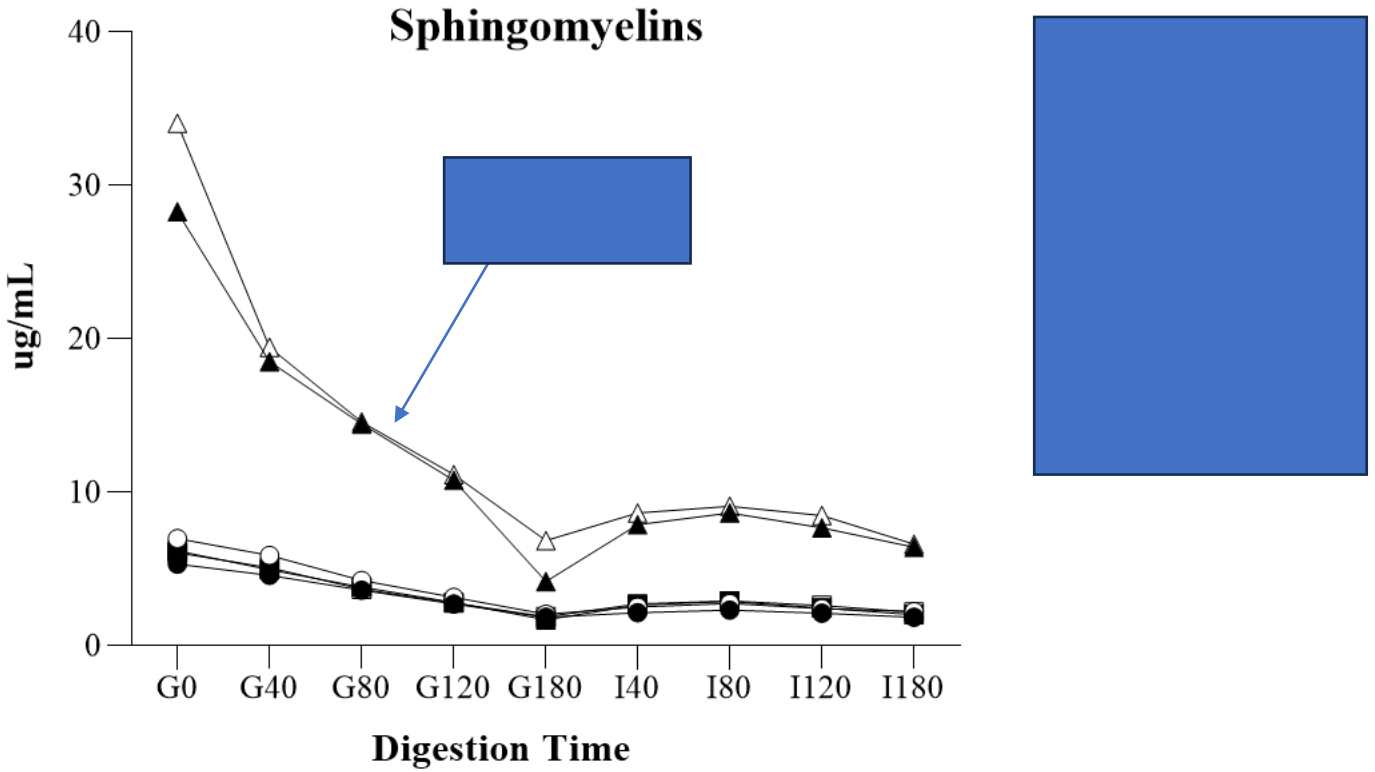






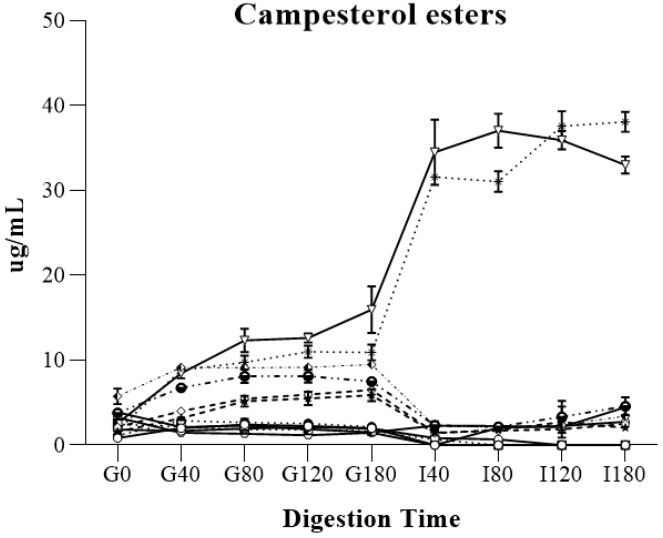
Unpublished data

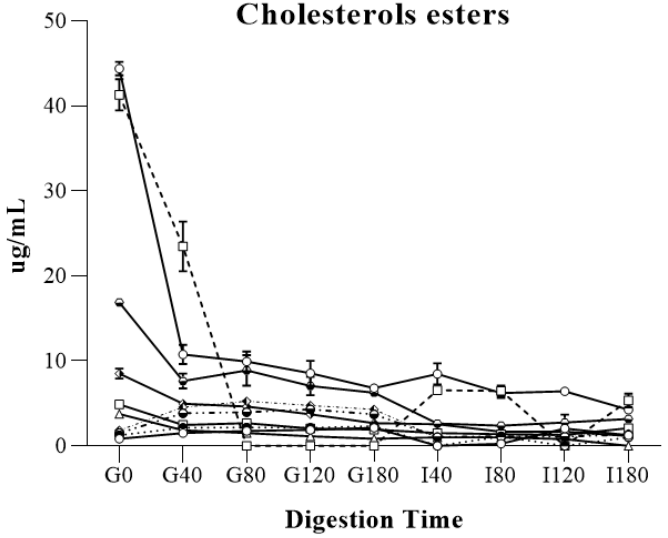




Dietary sphingomyelins are digested by intestinal alkaline SMase (Alk-SMase) and neutral ceramidase (N-CDase), and eventually hydrolyzed to ceramides, phosphocholine, sphingosine and fatty acids in the small intestine

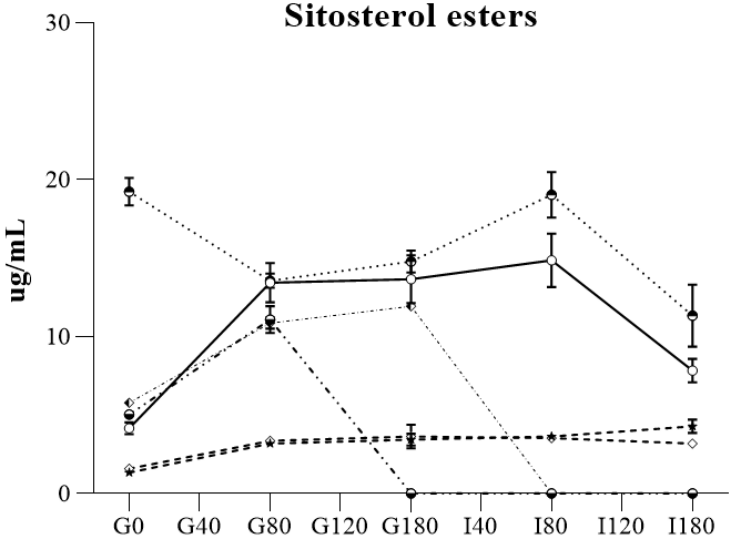
Unpublished data



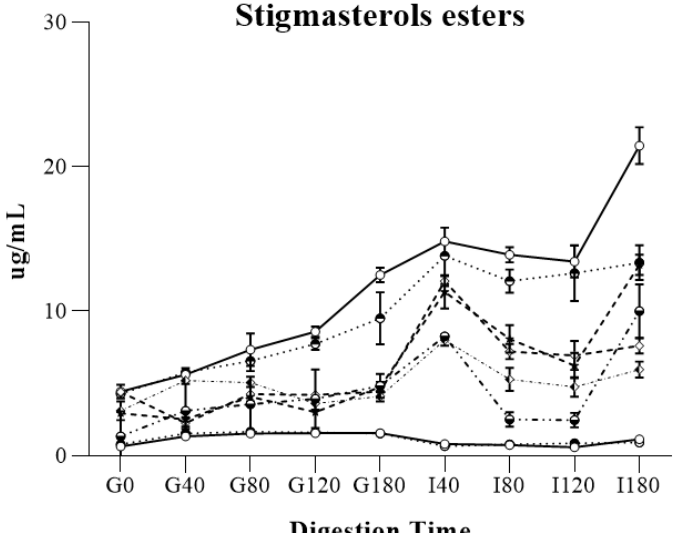


16:0-Glc-Cholesterol_S

Cholesterols and phytosterols



Unpublished data



TG percent digestibility

Molecular specie	Sheep %	Goat %
TG 24:0	96.5	96.7
TG 30:0	89.7	93.5
TG 36:0	68.5	76.1
TG 36:1	67.6	81.8
TG 38:0	61.3	68.7
TG 38:1	85.9	93.1
TG 40:2	100	100
TG 42:0	51,03	41,89
TG 42:1	75,68	60,13
TG 42:2	75,68	82,70
TG 44:0	25,08	43,04
TG 44:1	48,92	60,40
TG 44:2	100	100
TG 46:0	12,03	23,34
TG 46:1	19,98	35,46
TG 46:2	20,31	28,92
TG 46:4	0.0	0.0

Unpublished data

Fatty acid methyl esters (FAME) percent composition of the vegetable oil blend used in the production of sheep and goat IF (n=4).

Unpublished data

Fatty acid methyl esters (FAME) percent composition of sheep and goat IF (n=4).
t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Nd= not detected.

Unpublished data

Take home message

- We explored the fate of sheep and goat infant formula complex lipids using an in vitro dynamic digestion systems
- For the first time we were able to follow the hydrolysis/digestion of each complex lipid
- We demonstrated that the source of sheep milk lipids are almost equivalent to goat milk lipids
- Sheep milk can be exploited in UE for the formulation of IFs.