Liver functioning and ketosis management: how much depends on choline nutrition?

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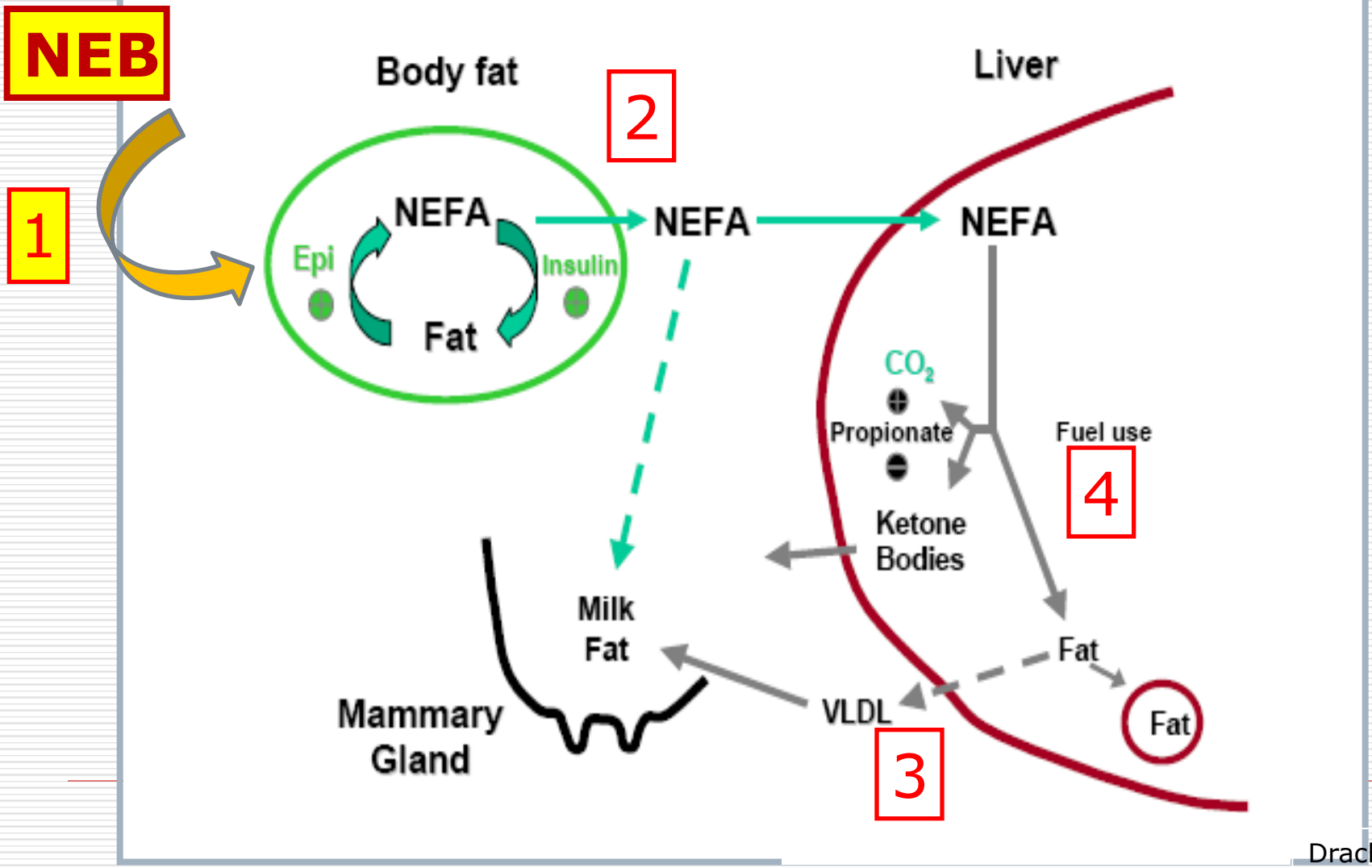
Introduction

- Choline is an essential nutrient that helps maintain a normal metabolism of fat in the liver, in addition to its role in cell structure and activity.
- Reduces the negative effects of hepatic lipidosis in transition cows.
- It is deficient in most dairy diets.

Shahsavari et al., 2016

Physiology of the negative energy balance



Drackley, 1999

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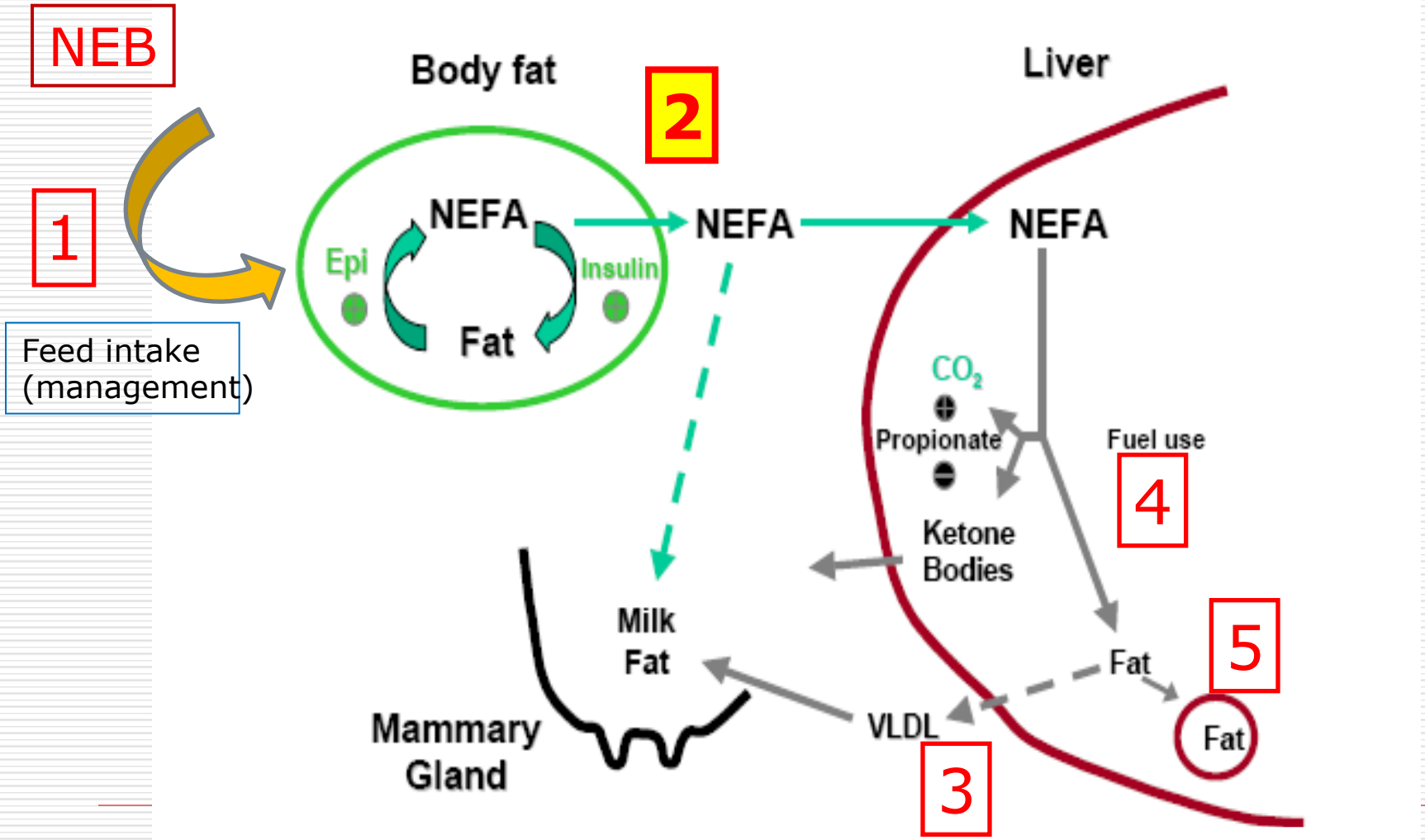

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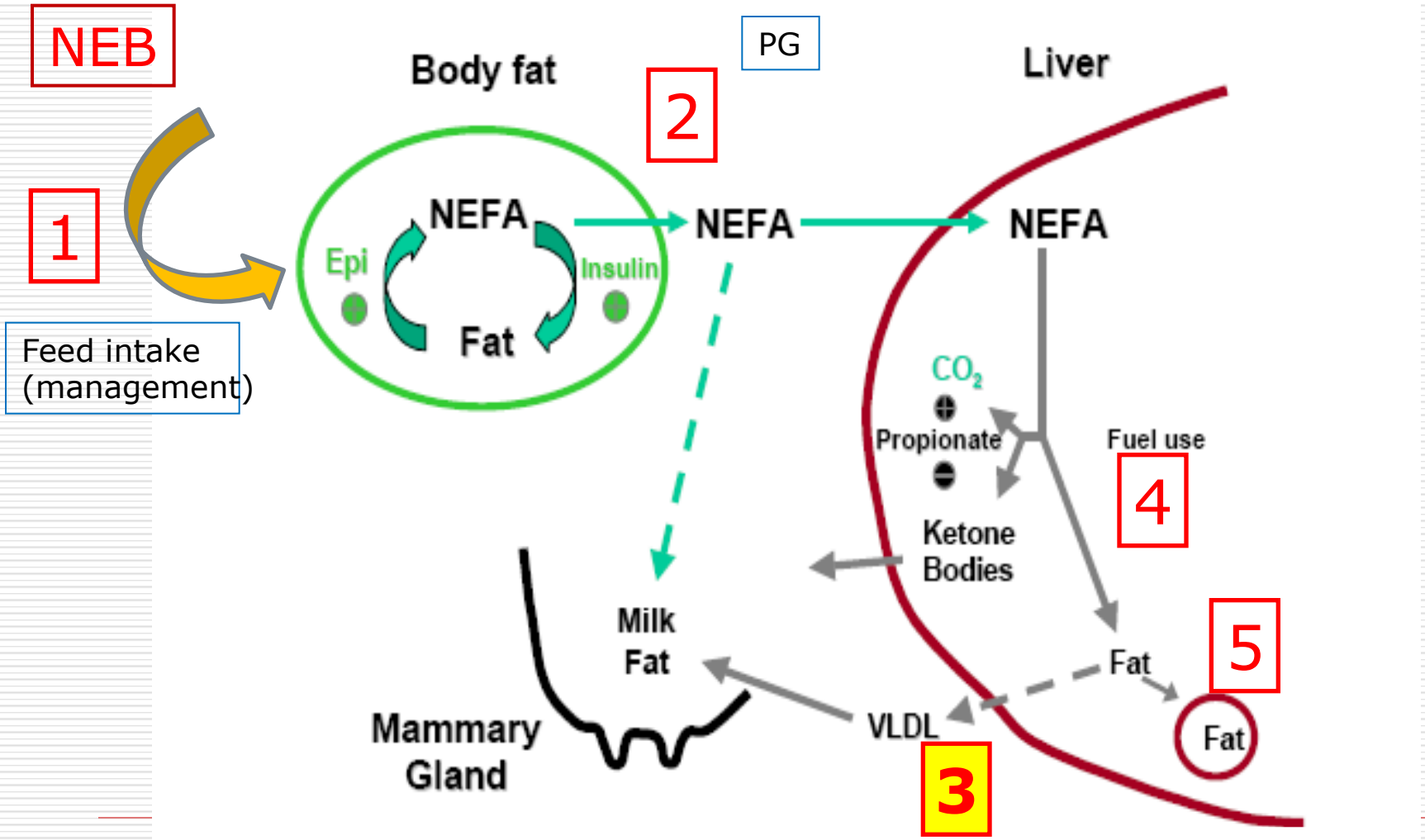

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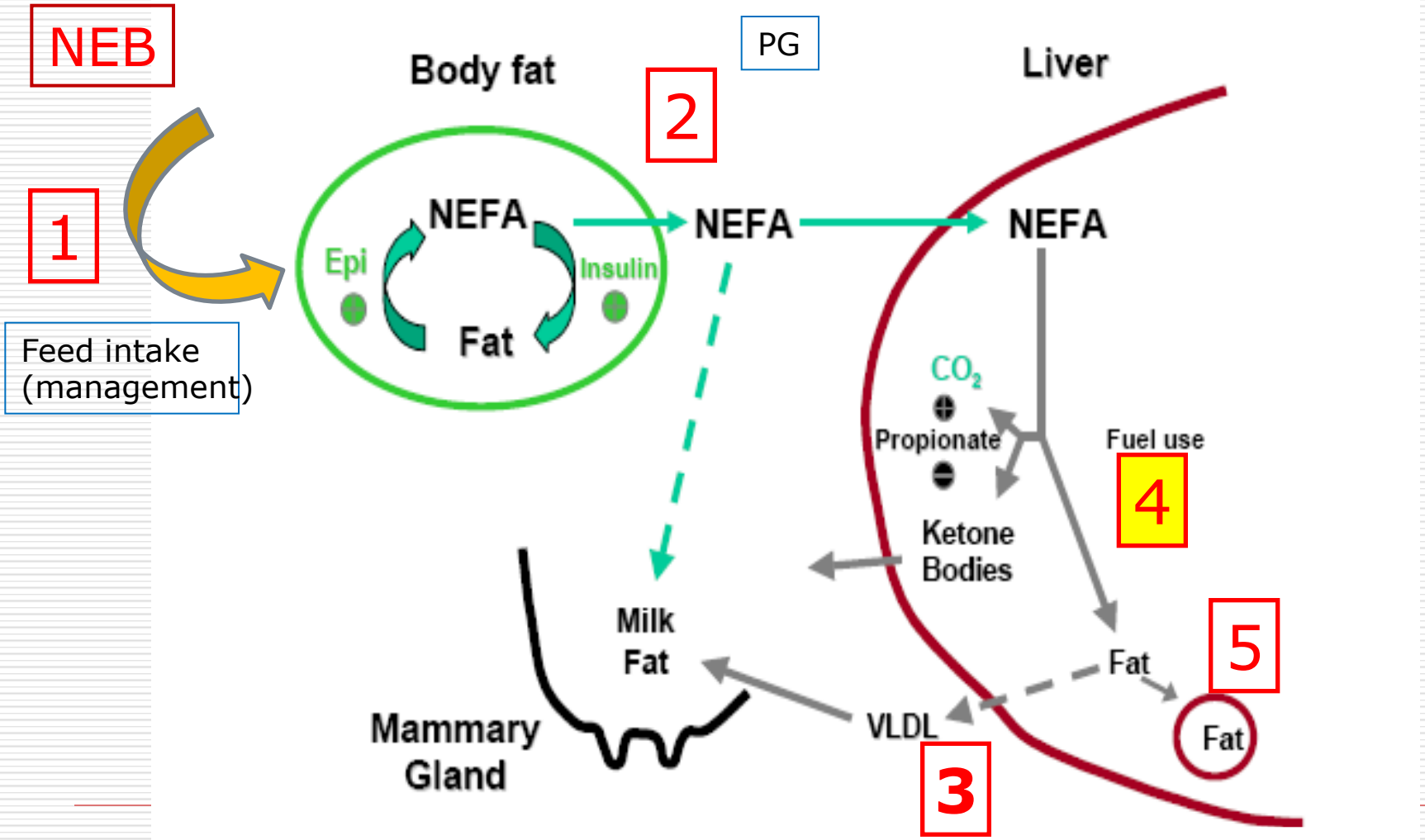


Drackley, 1999

Prevalence of subclínical ketosis

S.No.	Author	Continents	Total samples	Prevalence (%)
1	Dohoo and Martin, 1984	NA	2551	**12**
2	Al-Rawashdeh, 1999	Asia	1155	**25.6**
3	Sakha et al., 2007	Asia	90	**14.4**
4	Walsh et al., 2007	NA	[illegible]	**46.3**
5	Voyvoda and Erdogan, 2010	Asia	[illegible]	**33.3**
6	Asl et al., 2011	[illegible]	[illegible]	**63.3**
7	McArt et al., 2012	[illegible]	1717	**43.2**
8	Tehrani-Sharif et al., 2012	[illegible]	190	**18.4**
9	Garro et al., 2014	[illegible]	107	**10.3**
10	Ribeiro et al., 2013	[illegible]	771	**35.4**
11	Samiei et al., 2013	[illegible]	[illegible]	**12.2**
12	Suthar et al., 2013	[illegible]	5012	[illegible]
13	Berge and Vertenten, 2014	[illegible]	[illegible]	[illegible]
14	Krempaský et al., 2014	[illegible]	[illegible]	**18.5**
15	Compton et al., 2014	Oceania	[illegible]	**27.1**
16	Compton et al., 2015	Oceania	[illegible]	[illegible]
17	Vanholder et al., 2015	Europe	[illegible]	**47.2**
18	Sentürk et al., 2016	Asia	[illegible]	[illegible]
19	Biswal et al., 2016	Asia	[illegible]	**9.6**
20	Santschi et al., 2016	NA	[illegible]	**22.6**
21	Dubuc and Denis-Robichaud, 2017	NA	2520	[illegible]
22	Vince et al., 2017	Europe	841	[illegible]
23	Tatone et al., 2017	NA	165,749	**21**
24	Daros et al., 2017	SA	658	**20.7**
25	Hejel et al., 2018	Europe	1667	**29.3**
26	Brunner et al., 2019	Europe	164	**39**
27	Vallejo-Timarán et al., 2020	SA	249	**46**

27 publications

Prevalence 9.6 to 63.3 %

Loiklung et al-., 2022

Consequences of ketosis

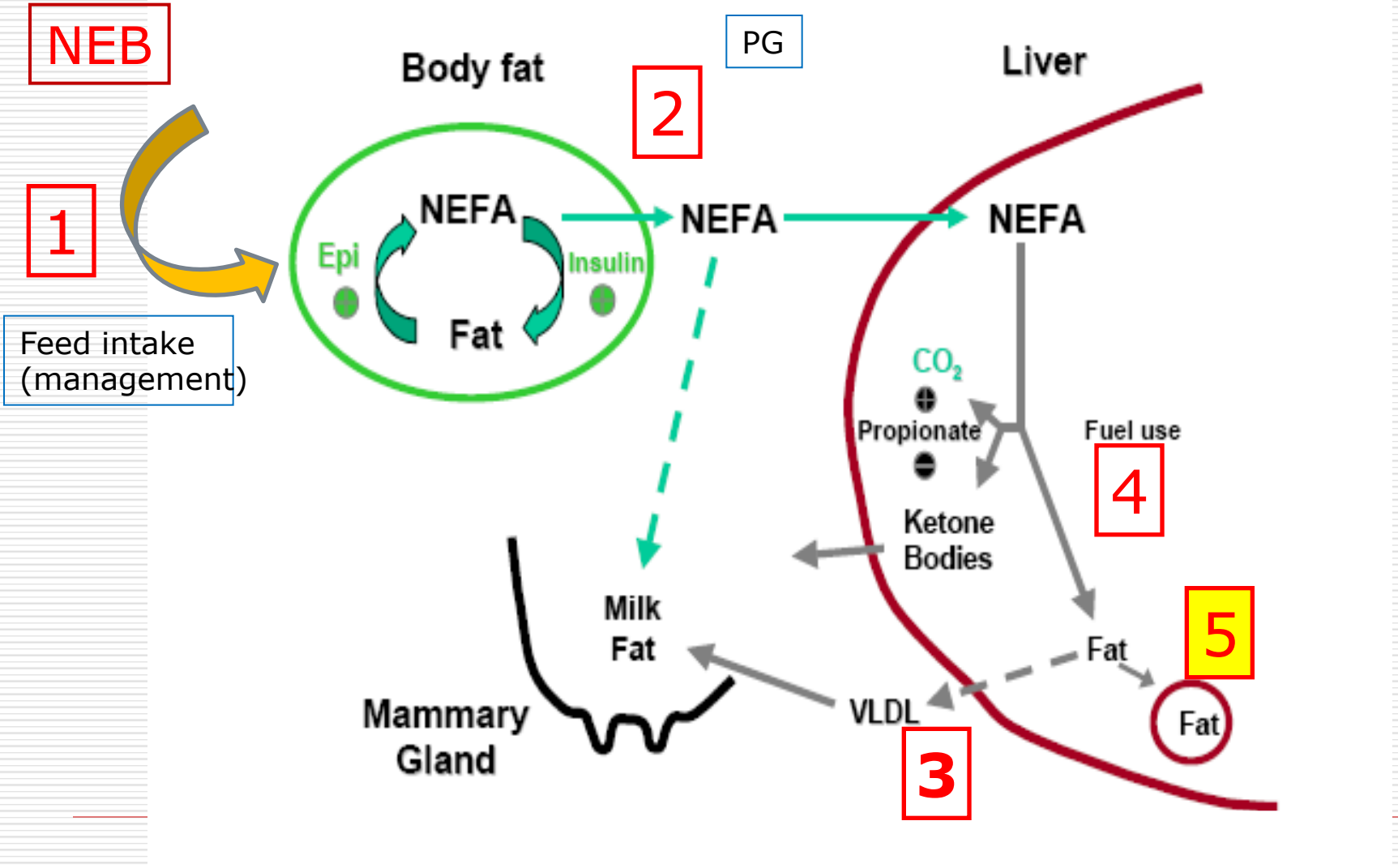
- ↓ 3 - 11% **Milk production** (n=10 studies)
- ↓ 22 al 50% **Fertility** (n=5 studies)
- ↑ risk of other dieseases
  - x6 Displaced abomasum (n=11 studies)
  - x3,6 Clinical ketosis (n=8 studies)
  - x2,75 Metritis (n=4 studies)

HIGH ECONOMIC LOSSES

↓ 339€/case

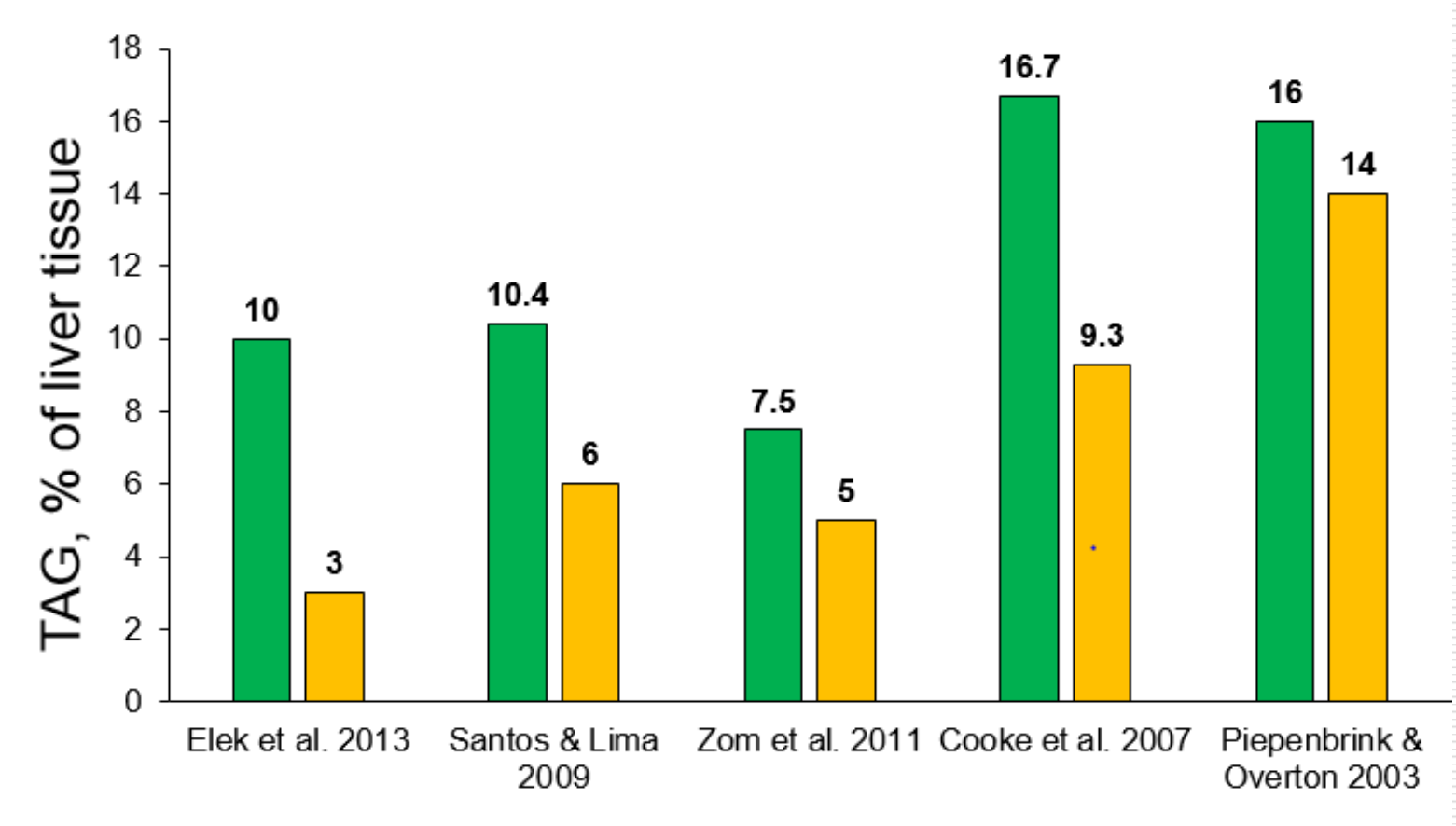
Marqués et al., 2019

Physiology of the negative energy balance

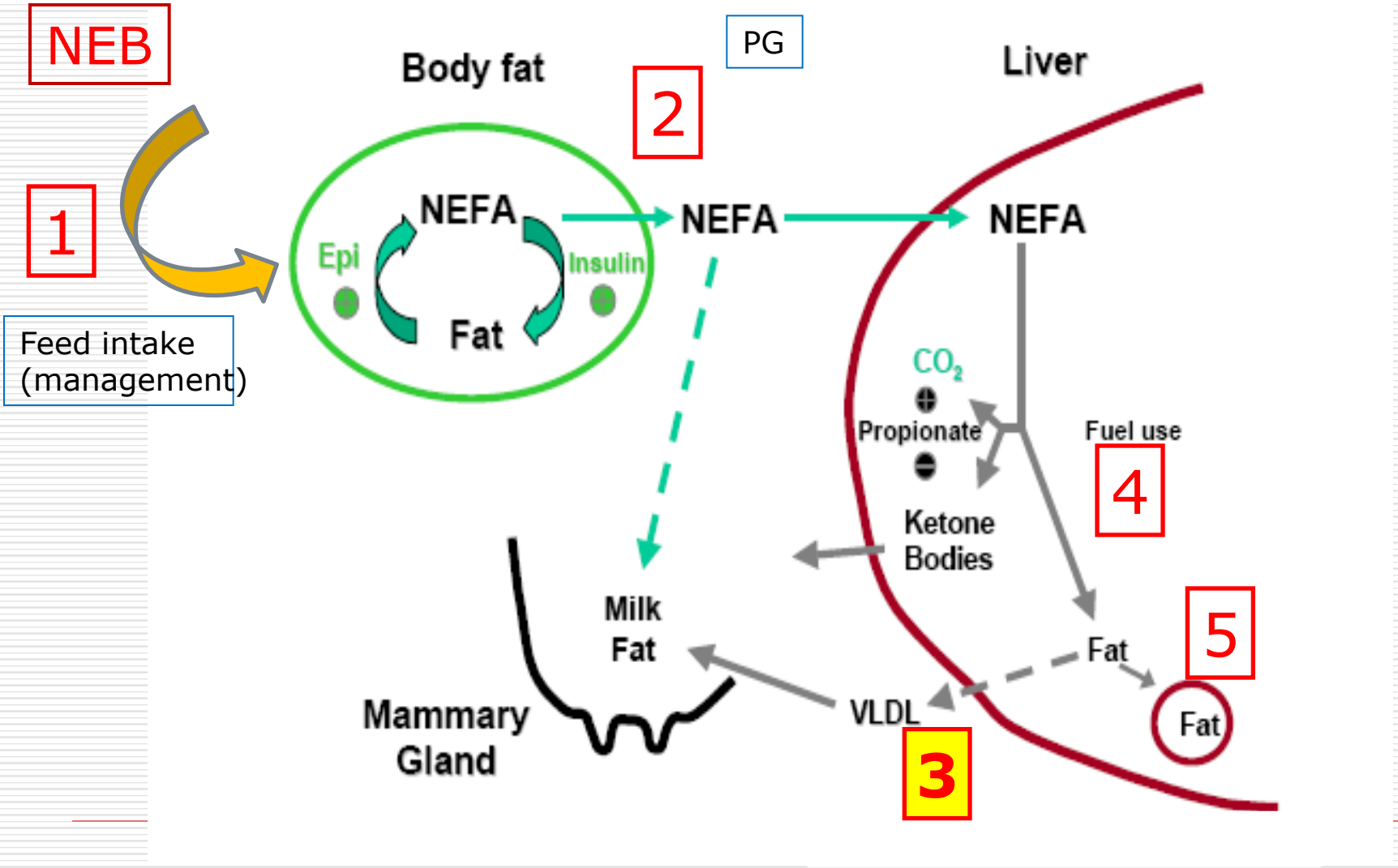


Drackley, 1999

Choline reduces the fat burden on the liver



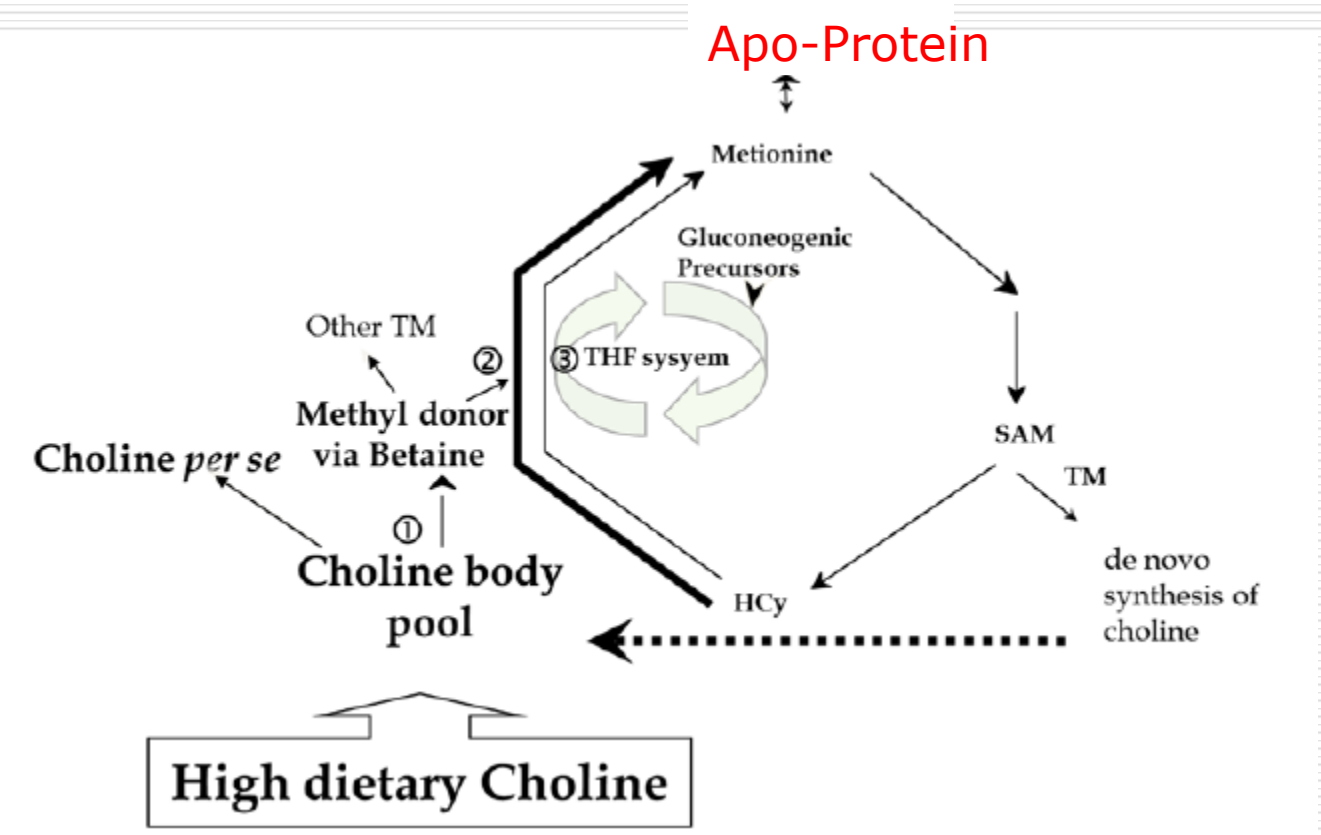
Physiology of the negative energy balance



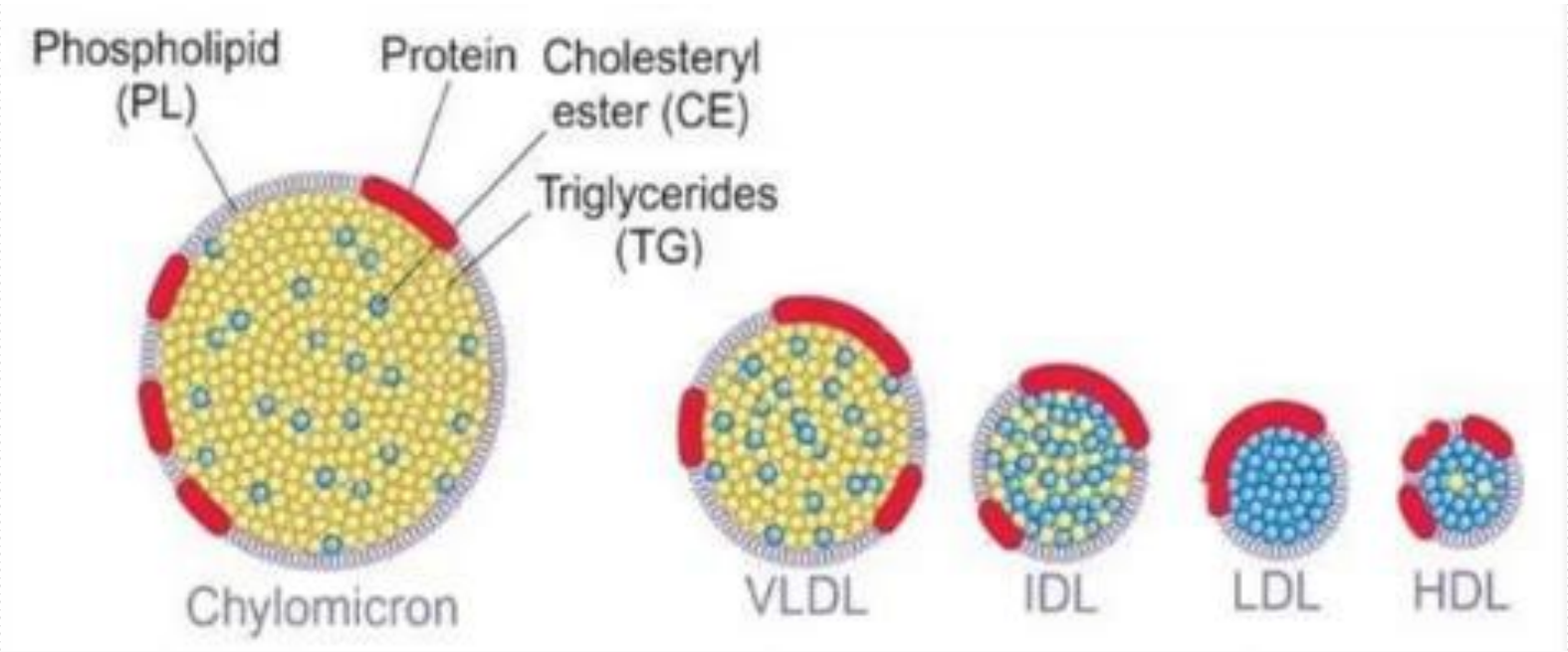
Drackley, 1999

Export of NEFA through VLDL

- Methyl group donor: Choline and Met



The Very Low Densisity Lipoproteins (VLDL)



Rodrigo-Fernández et al., 2018

Choline improves performance

Parameters	Choline ion (g/d)		Difference	P-value
	0	12.9		
Milk, kg/d	33.2	34.8	1.6	<0.001
ECM, kg/d	34.8	36.5	1.7	0.001
Milk Fat, kg/d	1.29	1.36	0.07	<0.001
Milk Proetin, kg/d	1.06	1.11	0.05	<0.001
Lactose, kg/d	1.65	1.66	0.01	0.003*
ECM/DMI	1.84	1.96	0.12	0.001

21 experiments

Up to 66 treatment means

1313 prepartum parous cows

Research data search from 1984 to 2018

Prepartum avg. RPC feeding 22±6.0 day

Postpartum avg. RPC feeding 57.5±42.2 day

Arshad et al., 2020

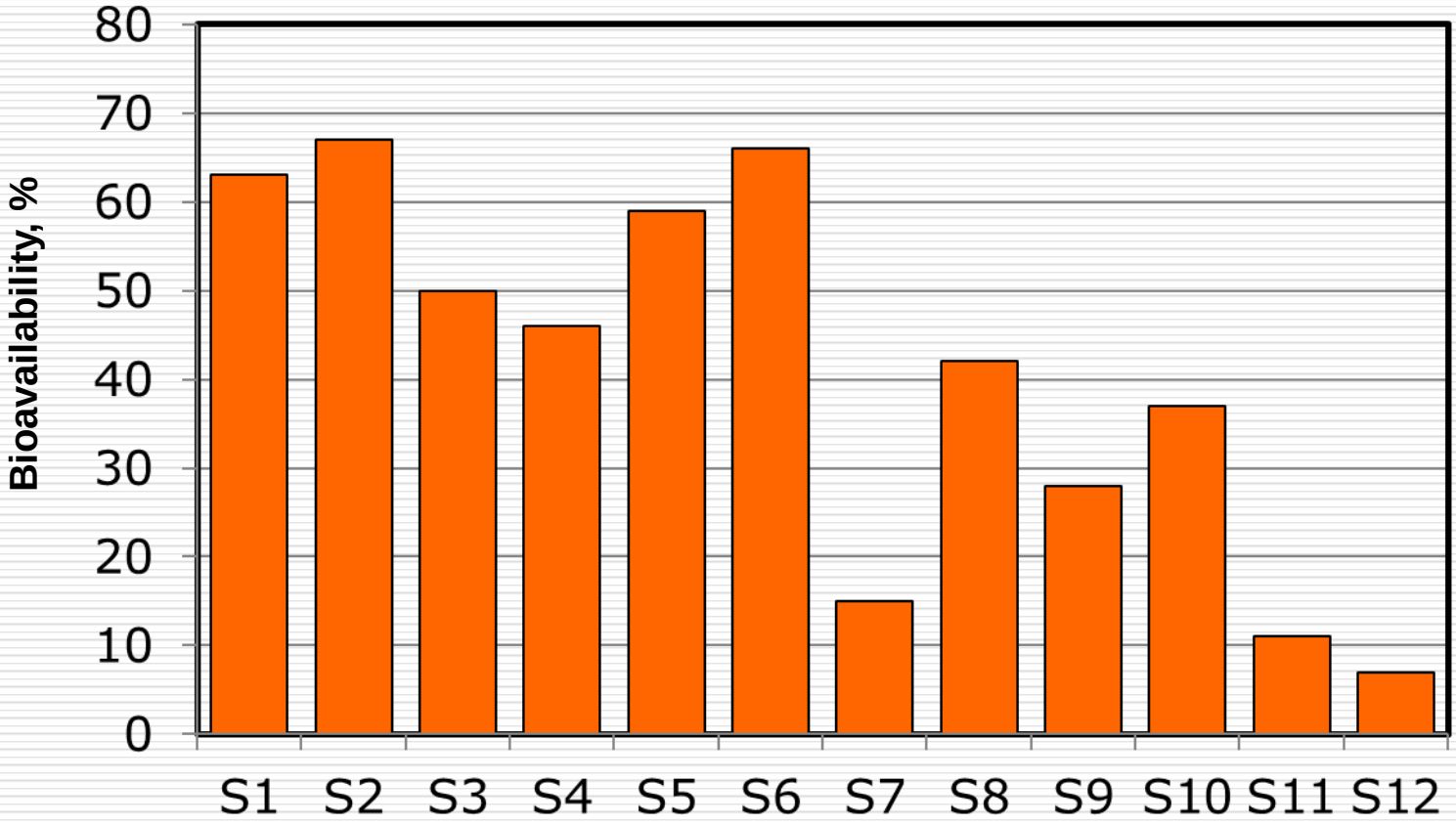
Summary 1

- Negative energy balance in an essential part of energy nutrition postpartum
- The incapacity of the liver to manage excess fat mobilization may result in ketosis
- Ketosis is highly prevalent and costly in dairy farms
- Although ketosis can be prevented by reducing fat mobilization, helping the liver to export NEFA is most efficient
- Choline plays an essential role in helping fat export from the liver to the mammary gland, enhancing performance

Choline needs protection

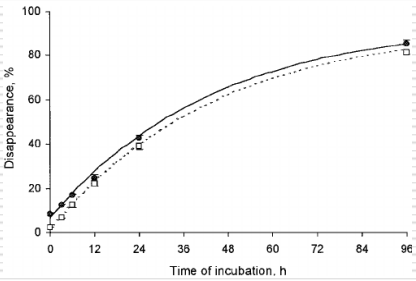
- Choline is highly degradable in the rumen and needs to be fed protected
- Variability among products is high

Variation among rumen protected AA (in situ - in vitro)

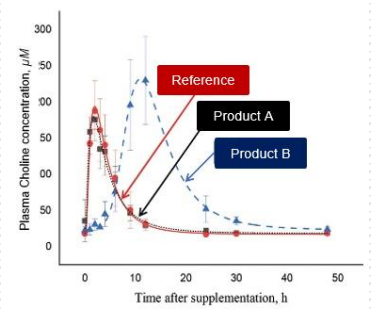


Methods of bioavailability evaluation

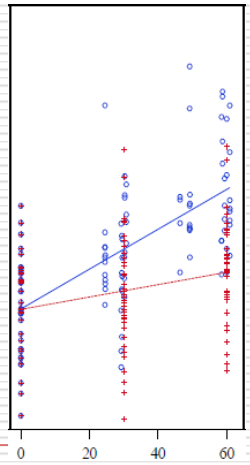
- In situ / in vitro (nylon bag)



- Plasma choline area under the curve



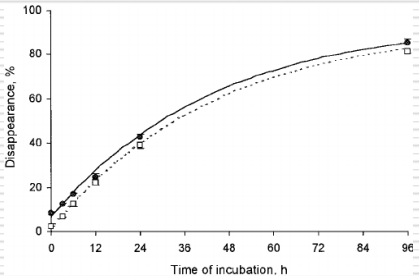
- Plasma choline dose-response technique



- Production performance

In situ / in vitro evaluation

- **In situ rumen degradaton and in vitro intestinal digestion**
- **Free-plasma choline** (Whitehouse et al. 2016)
- **Area Under the Curve (AUC)**
- **Lactational performance**



Material and methods

- **Products**
  - Product A
  - Product B
  - *Product C

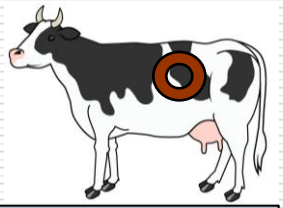
*CholiGEM™, Kemin Animal Nutrition and Health

Material and methods

- **In situ procedure (rumen)**
  - Cannulated dairy cows.
  - 2.02 g/bag in duplicate.
  - Incubations at 0-2-4-8-16-24-48 h.
  - Two periods.

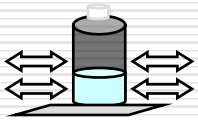
Intestinal digestibility

- 3-Step intestinal digestion *(Calsamiglia and Stern, 1995; Gargallo et al., 2006).*



12 h Ruminal Incubation

Wash + Freeze

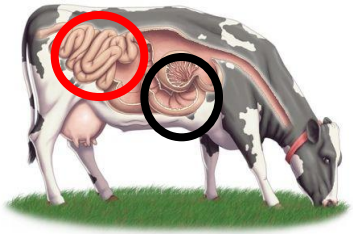


0.1 N HCl + 1 g/L pepsin (39ºC, pH 1.9, 1 h)

Wash

0.5 M KH2PO4 + 50 ppm thymol + 3 g/L pancreatin (39ºC, pH 7.75, 24 h)

Wash + Dry

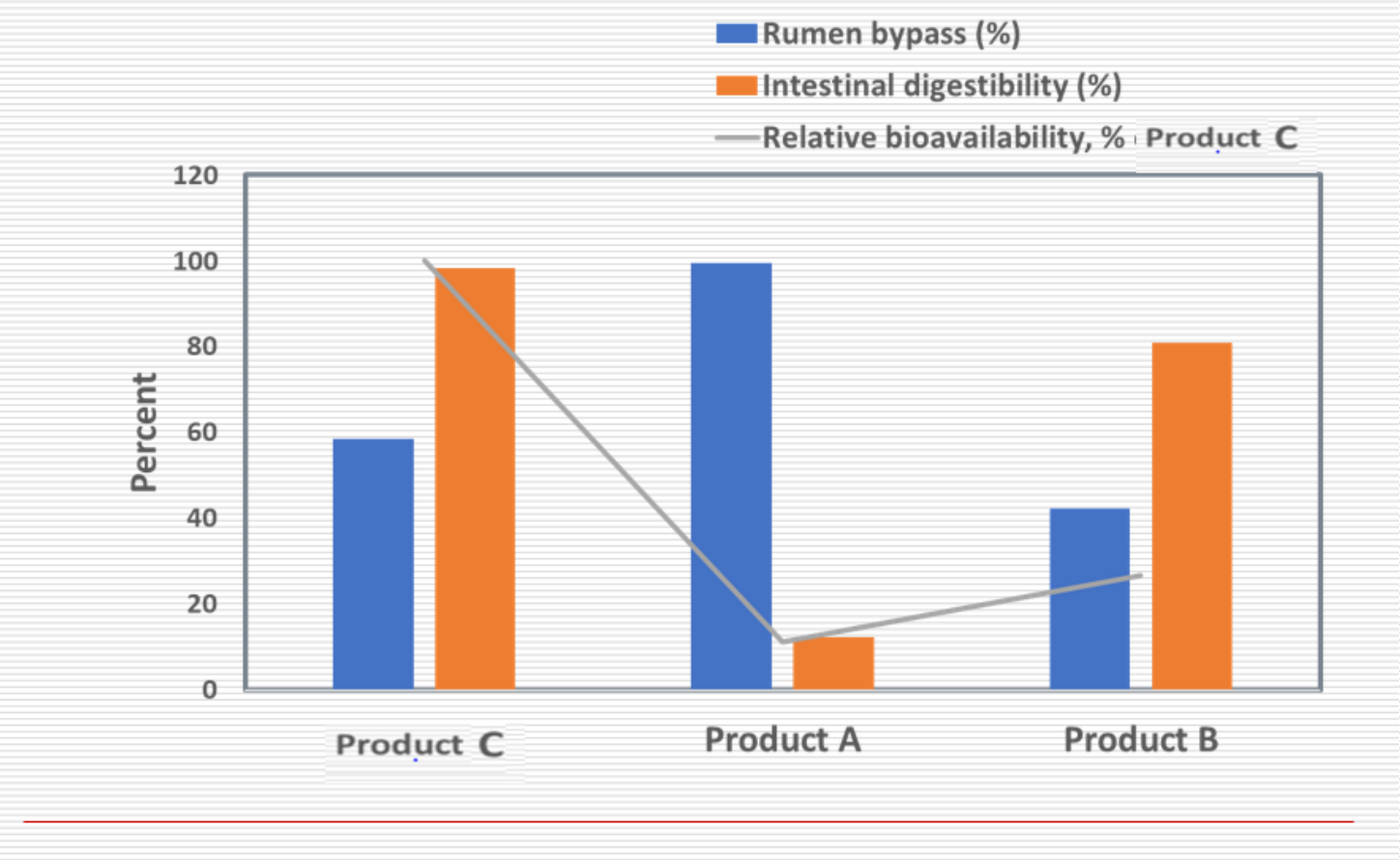


Intestinal digestion

Relative bioavailability was calculated

RBV = N not degradable (%) * digestibility (%)

Results : Relative bioavailability

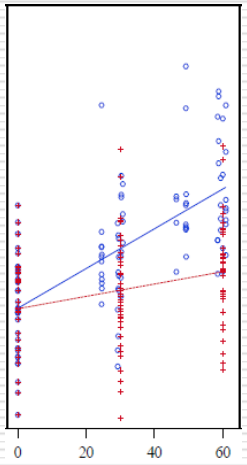


Summary 2

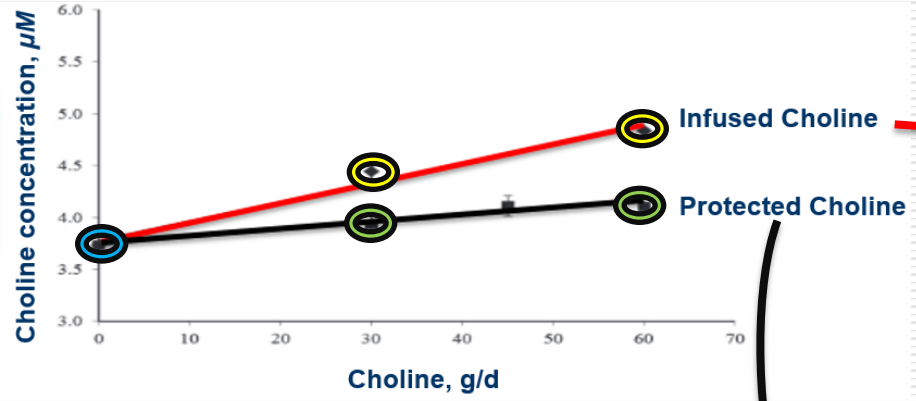
- There are relevant differences in ruminal degradability and intestinal digestibility of Choline among different rumen protected products that have an important impact on overall bioavailability.
- Evaluation of rumen protected products require the evaluation of both ruminal degradability and intestinal digestibility.
- When comparing with Product C, relative bioavailability of Product A stands at 11.2 % (lowest) and Product B at 26.8% (intermediate).

Dose response study : Plasma free-choline

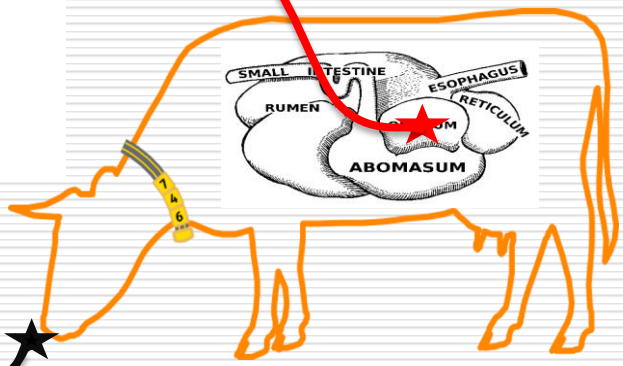
- In situ rumen degradation and in vitro intestinal digestion
- Area Under the Curve (AUC)
- **Dose response study- plasma free-choline (Whitehouse et al. 2016)**
- Lactation performance



Plasma free choline dose-response method



The ratio of slopes indicates bioavailability



Whitehouse et al., 2017

Abomasal infusion tool (Gressley et al., 2006)

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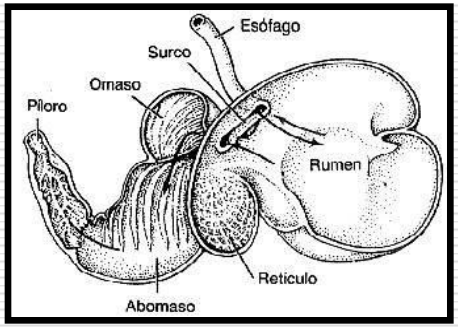
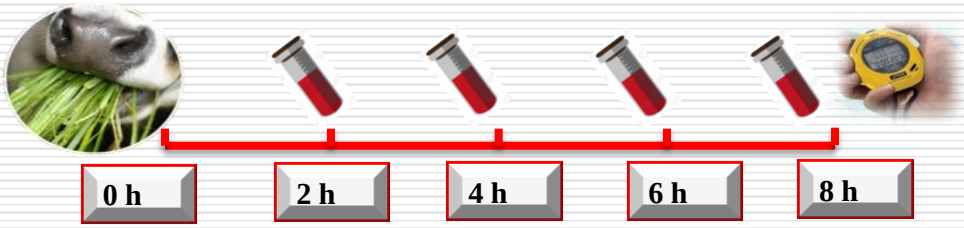


Samples and analyses

3. Blood sample



- Centrifuged at 2000 × g for 20 min at 4ºC to isolate plasma for Choline analysis
- Plasma Choline analysis with HPLC

Available data

- Three studies conducted:
  - DeVeth et al., 2016
  - Potts, 2019
  - SNIBA-UAB, 2021
- Common findings
  - Bioavailability determined from choline and its major metabolites (betaine and P-choline) as markers
  - Overall bioavailability is low
  - The method, a gold-standard for amino acids, does not work for choline bioavailability (inadequate markers)

Protected choline bioavailability using the AUC method

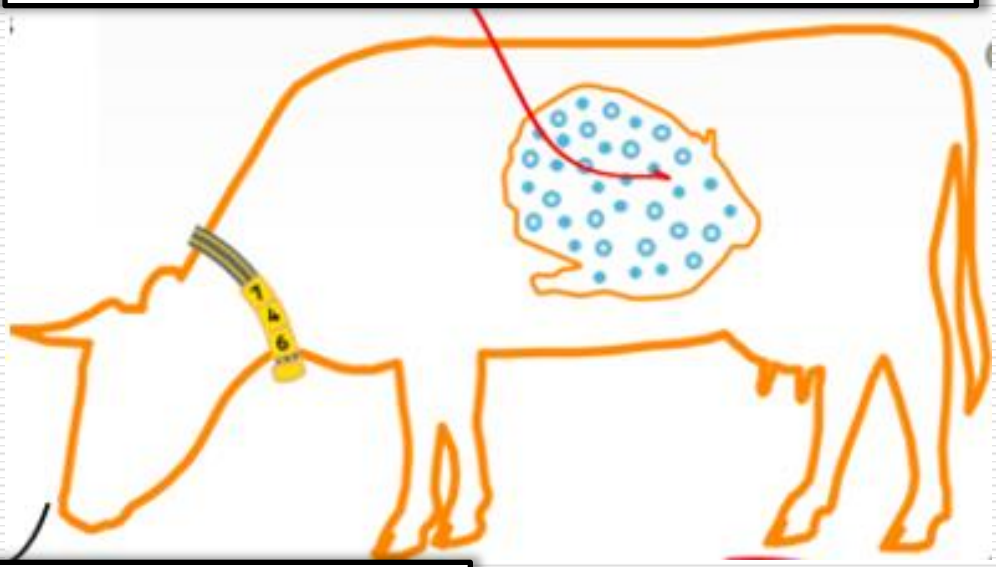
- In situ rumen degradaton and in vitro intestinal digestion
- Free-plasma choline (Whitehouse et al. 2016)
- **Area Under the Curve (AUC)**
- Lactational performance

Material and Methods

- Six cannulated multiparous lactating cows (603 kg BW; 215 DIM; 32 kg/d of milk) were used.
- Cows were randomly assigned to a switchback design to evaluate the plasma kinetics and the relative bioavailability of two RPC.
- Each period consisted of a single dose of Product C and Product A, followed by 3-d sampling.

The Area Under the Curve method

Infuse pure choline chloride in small intestine as a reference to see in blood choline concentration

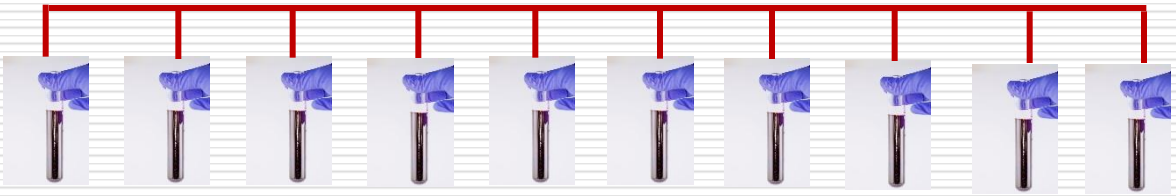


36 g/d

Give encapsulated choline chloride product to the cow and compare

Samples and analyses

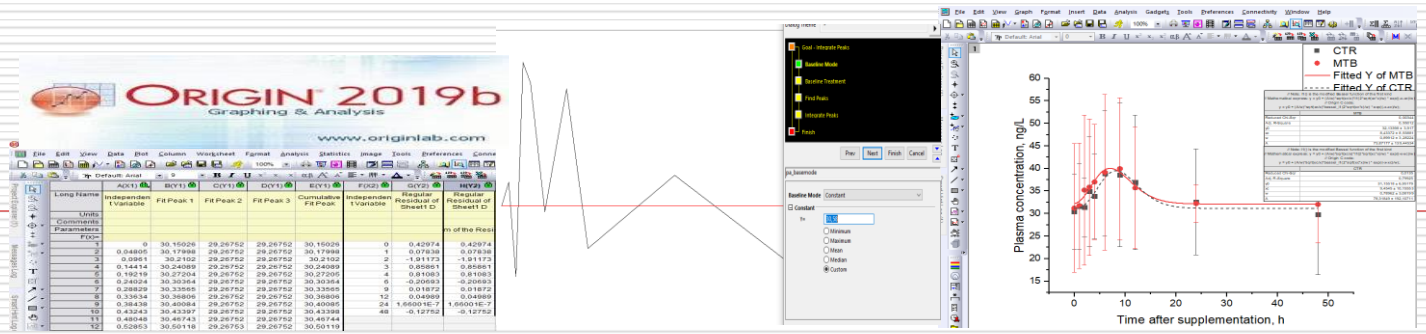
- Blood samples were collected at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30, and 48 h after treatment administration



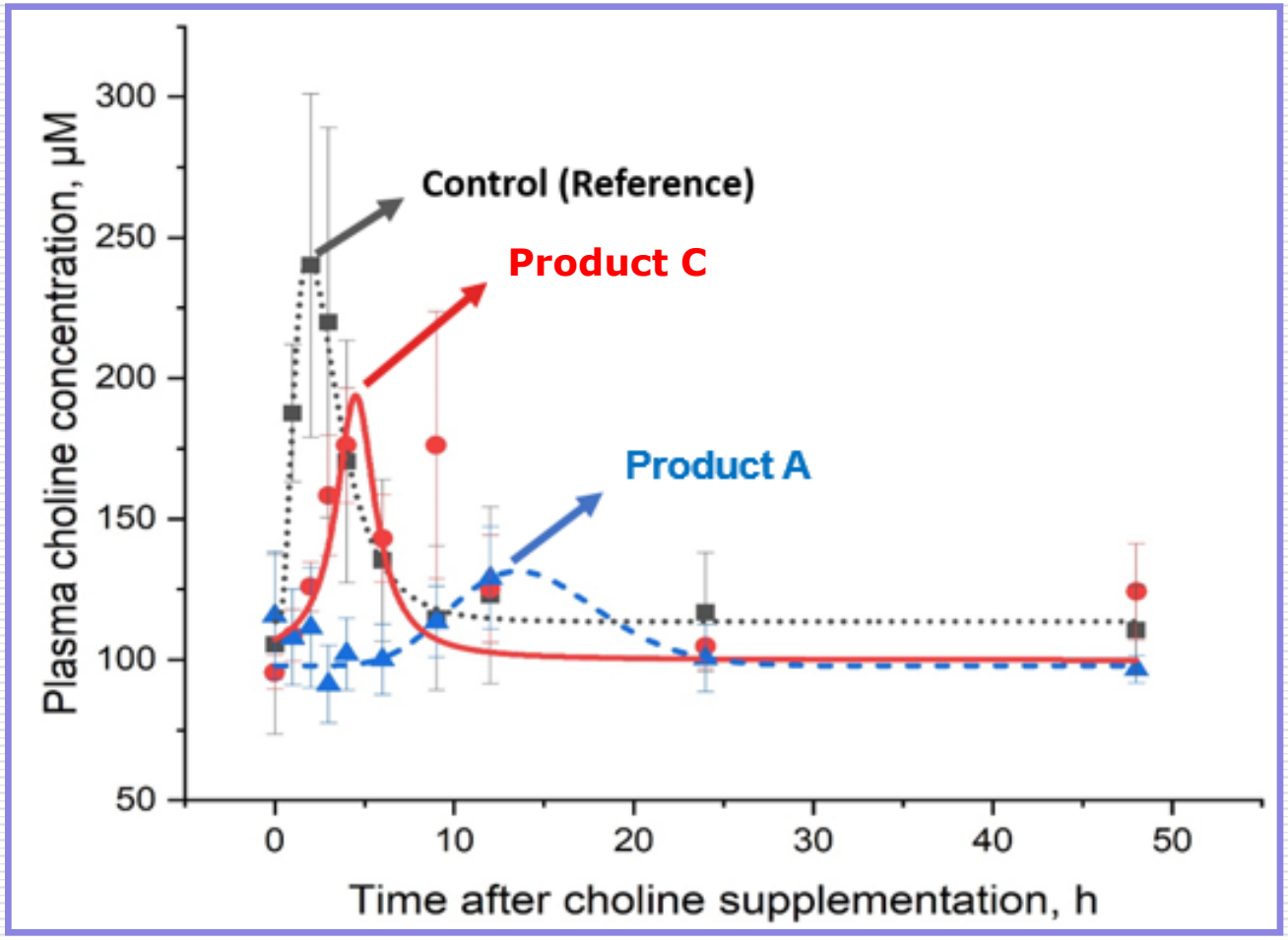
- The blood is centrifuged at 2000 rpm × g for 20 min at 4 °C to isolate the plasma.
- Plasma was analyzed for choline and betaine with HPLC.

Samples and analyses

- **Data treatments and analysis**
  - Data adjusted to the best fitted curves using OriginLab software.
  - Kinetic parameters: basal concentration (Cbasal), maximal concentration (Cmax), time at maximal concentration (Tmax) and area under the curve (AUC).
  - Results were analyzed with the Glimmix model of SAS.



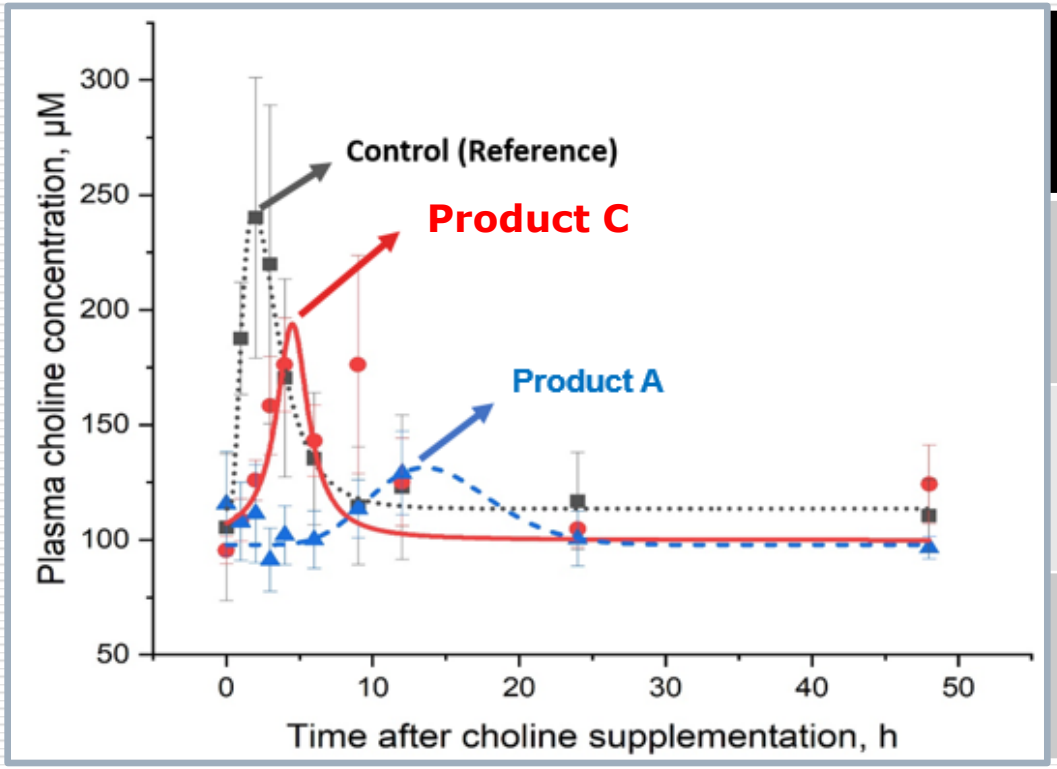
Results


Plasma choline concentration, µM
300
250
200
150
100
50
0
10
20
30
40
50
Time after choline supplementation, h
Control (Reference)
Product C
Product A

Results

Items	Product C	Product A	SEM	P-value
Cbasal, µM	96.8	90.7	0.57	0.09
Cmax, µM	227.1	155.2	10.11	0.22
Tmax, h	5.4	10.9	0.87	0.26
AUC, ^{5}AU	1279 [a]	503.3 [b]	33.10	> 0.05

Bioavailability



Choline Source	Bioavailability, %
Infused pure Choline	100
Product C	90
Product A	40

Conculsions

- Subclinical ketosis is highly prevalent in dairy herds
- The best strategy to resolve the problem and enhance performance is to help the liver export NEFA
- Choline plays an essential role in helping export NEFA as VLDL into the mammary gland

Conculsions

- Choline is highly degradable in the rumen and needs to be protected from rumen degradation, yet being digestible in the small intestine
- There is large variations among comercial products in their bioavailability: evaluating rumen degradation and intestinal digestion is essential. The AUC method provides a fair an unbiassed value for the true relative bioavailability of RP-Choline
- Supplementing dairy cows with a well rumen-protected choline results in relevant production responses (n=36; 2 kg/d)