



# In vitro and in vivo approaches for investigating gastro-intestinal disintegration of infant formula by the neonate

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# ***In vitro* and *in vivo* approaches for investigating gastro-intestinal disintegration of infant formula by the neonate**

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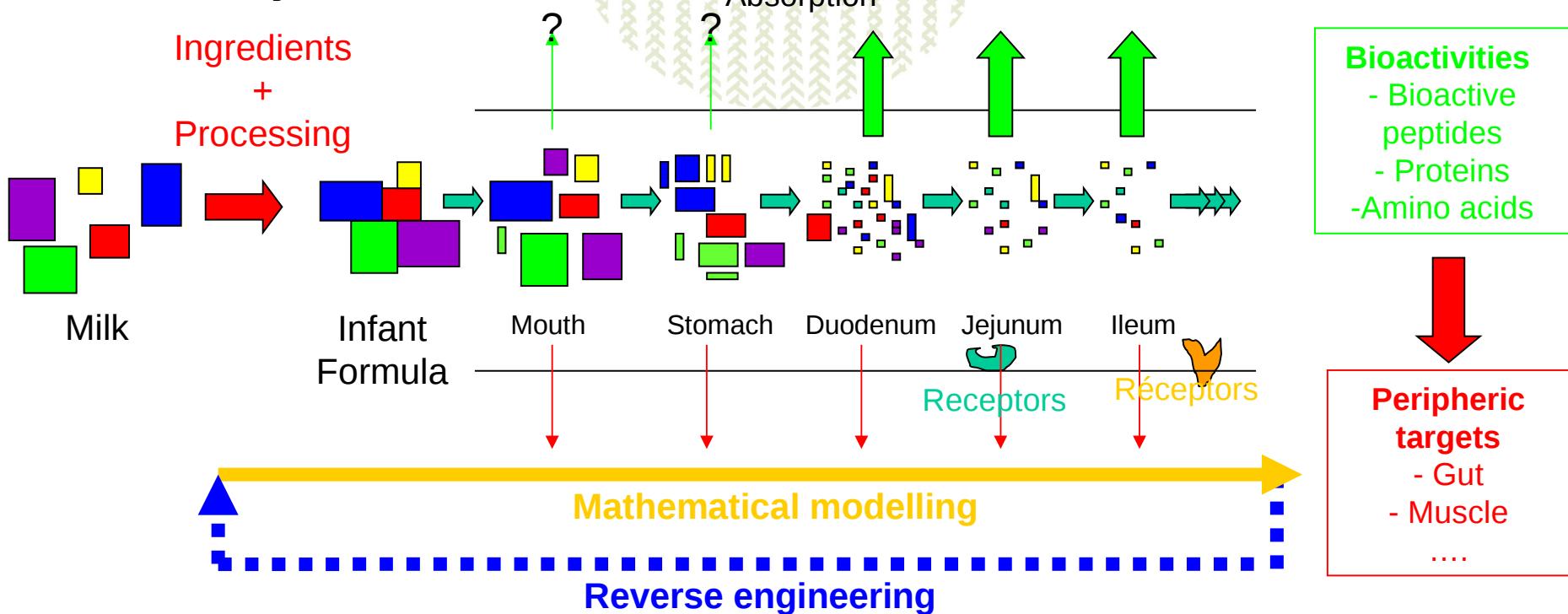
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# General objectives



- To understand the mechanisms of disintegration of infant formula and their proteins in the digestive tract
- To determine the impact of the bioactives released during digestion on the infant health
- To model these phenomena in order to develop a reverse engineering approach

# Does heat treatment of milk affect caseins digestibility?

☞ CNs = major allergens in infant whereas highly sensitive to digestion



Step 1 : Dissolution in milliQ water

Skimmed ultra-low-heat powder

25% DM

35% DM

ULH Milk

ULH Milk

Step 2 :  
Heat  
treatment

80°C/20 s.

105°C/60 s

85°C / 3 min

L.A

L.B

L.C

80°C/20 s

105°C/60 s

85°C / 3 min

L.E

L.F

L.G

Step 3 :  
Spray drying

A

B

C

E

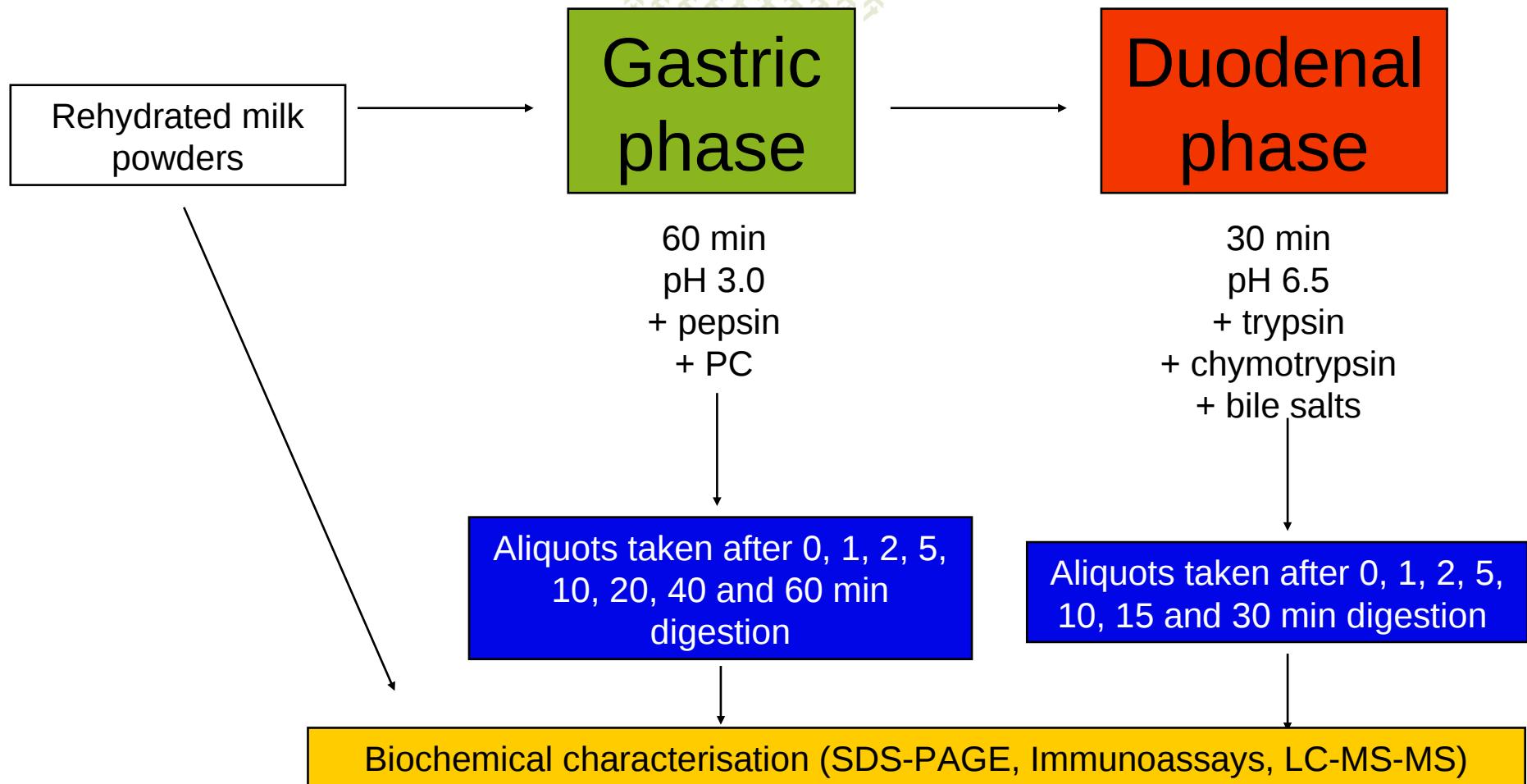
F

G

T

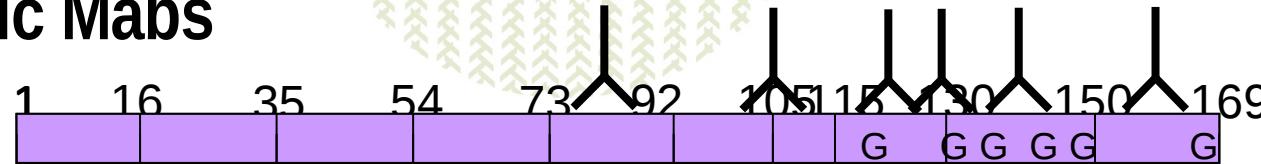
Digestion

# Infant gut Model (Dupont et al. Mol Nutr Food Res 2010, 54, 767-780)

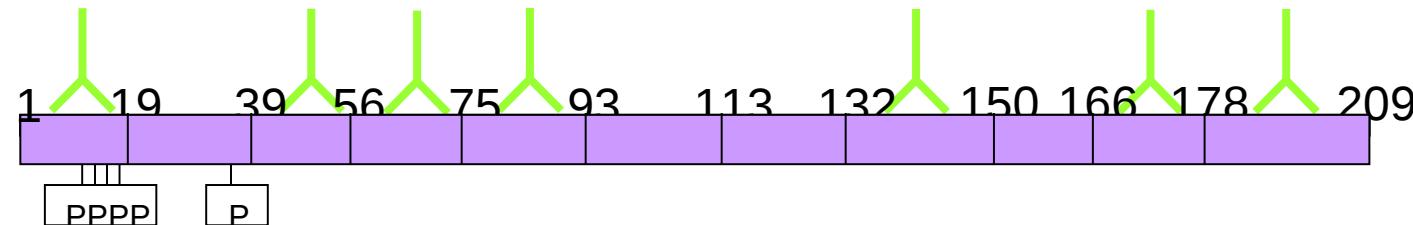


# Identification of epitopes resistant to digestion by ELISA using specific Mabs

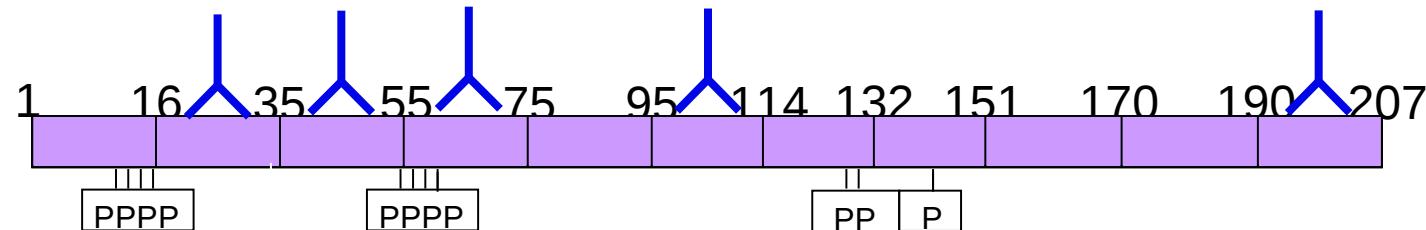
$\kappa$ -casein



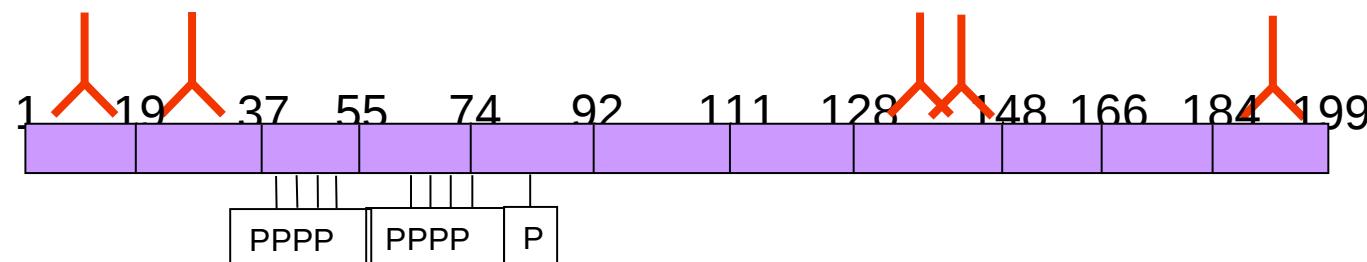
$\beta$ -casein



$\alpha s_2$ -casein



$\alpha s_1$ -casein



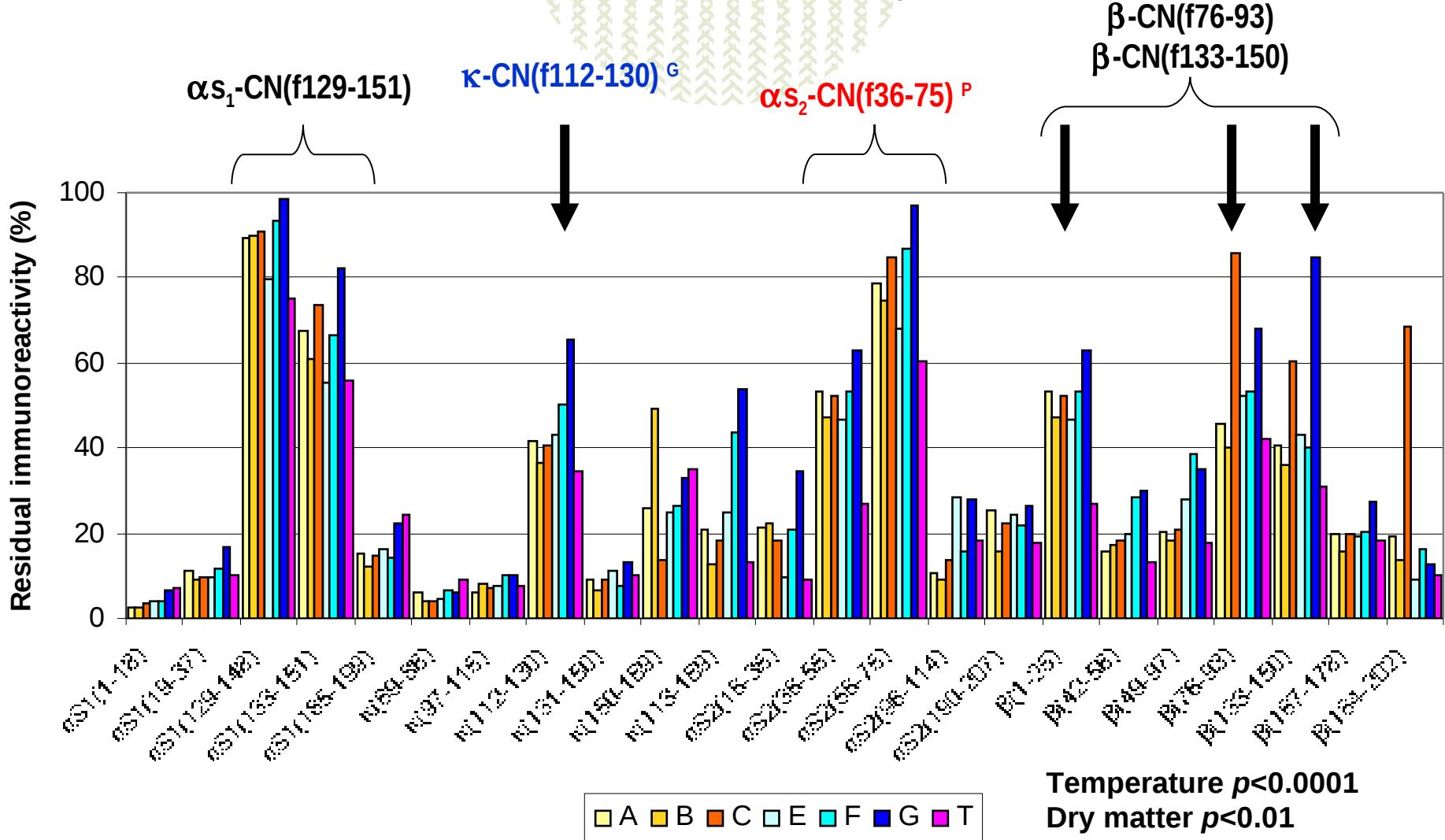
For more information, see poster P26

DIET

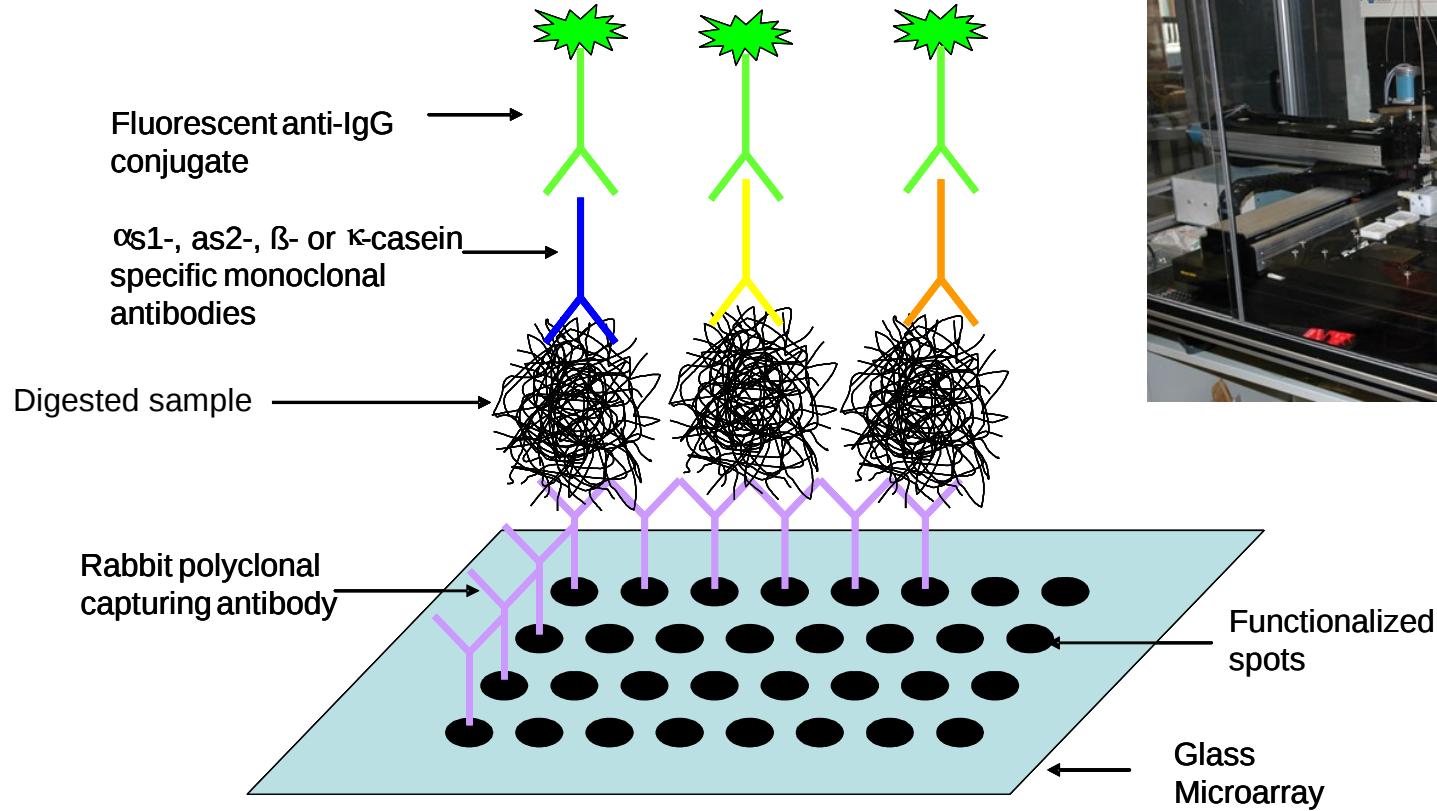
AGRICULTURE

ENVIRONMENT

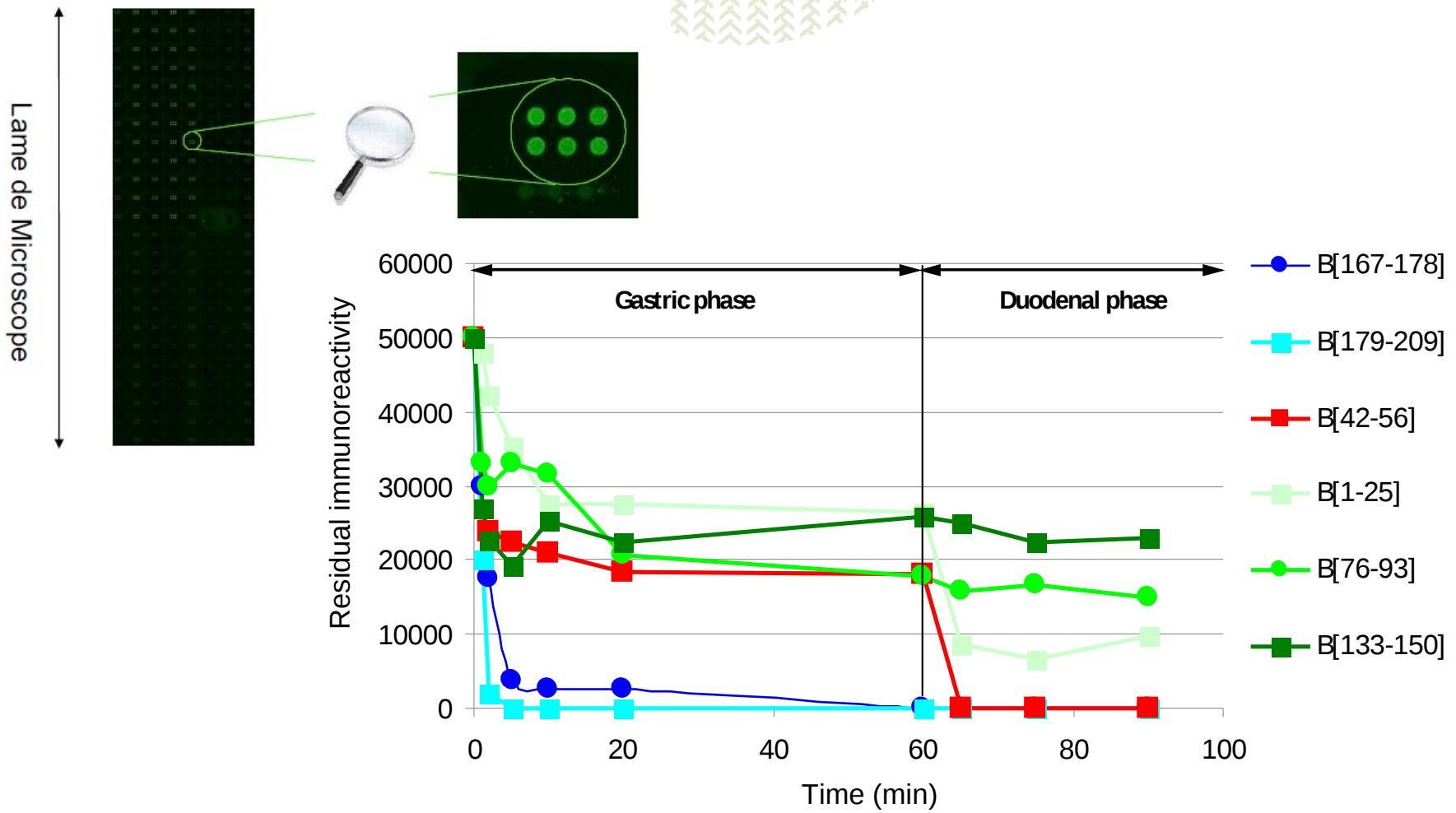
# Residual immunoreactivity of casein fragments



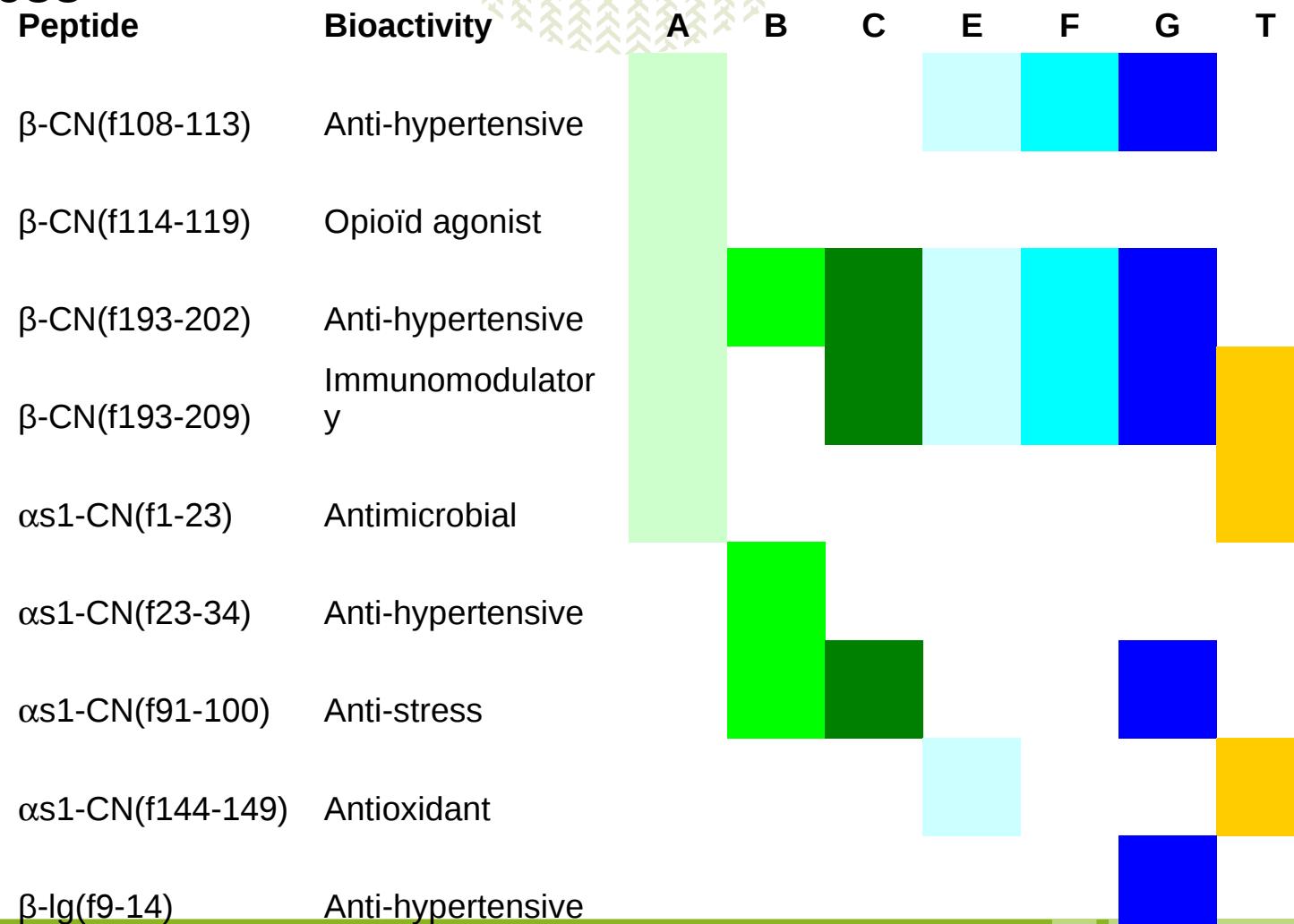
# Antibody array technology



# Kinetics of $\beta$ -casein digestion depend on the area studied

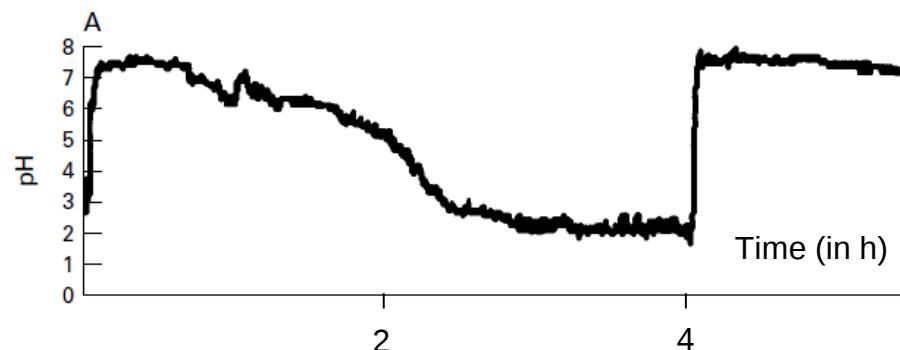


# Pattern of bioactive peptides resistant to digestion depends on the process



# Conclusion 1

- Heat treatment increases caseins resistance to digestion (**Dupont et al. 2010, Mol Nutr Food Res 54 ; Dupont et al. 2010, Food Dig, in Press**)
- Increased resistance is associated to the formation of heat-induced aggregates between casein micelles and whey proteins
- Limits of the study:
  - Model matrix with no lipids
  - Static *in vitro* digestion model that does not consider the flow of the food and pH variations



- Need to develop dynamic *in vitro* and *in vivo* approaches

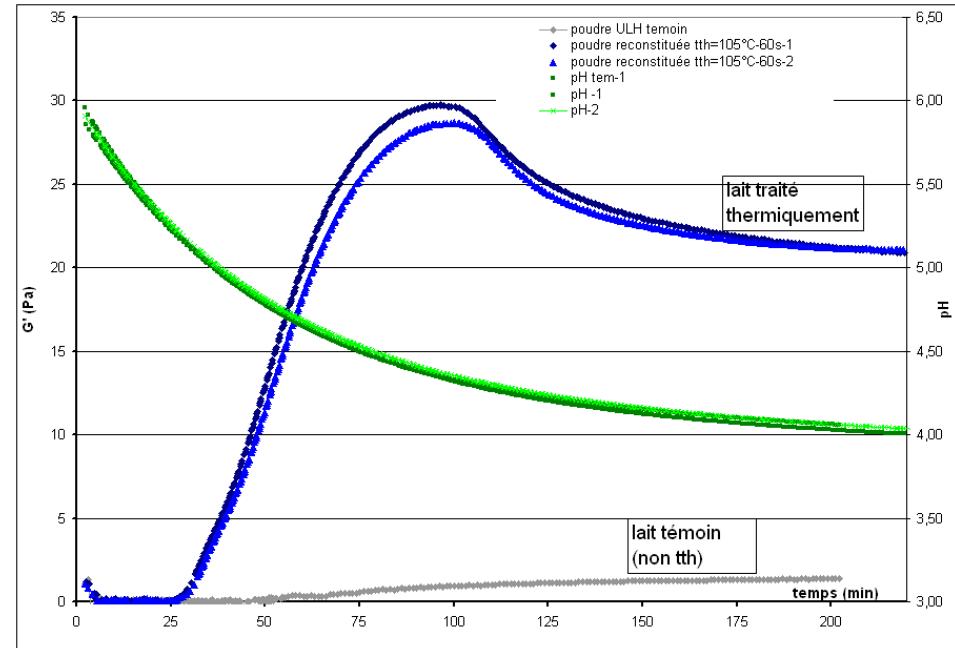
# Predicting matrix viscosity in gastric conditions

Simulation of pH decrease in the stomach using gluconodeltalactone (GDL) in a rheometer



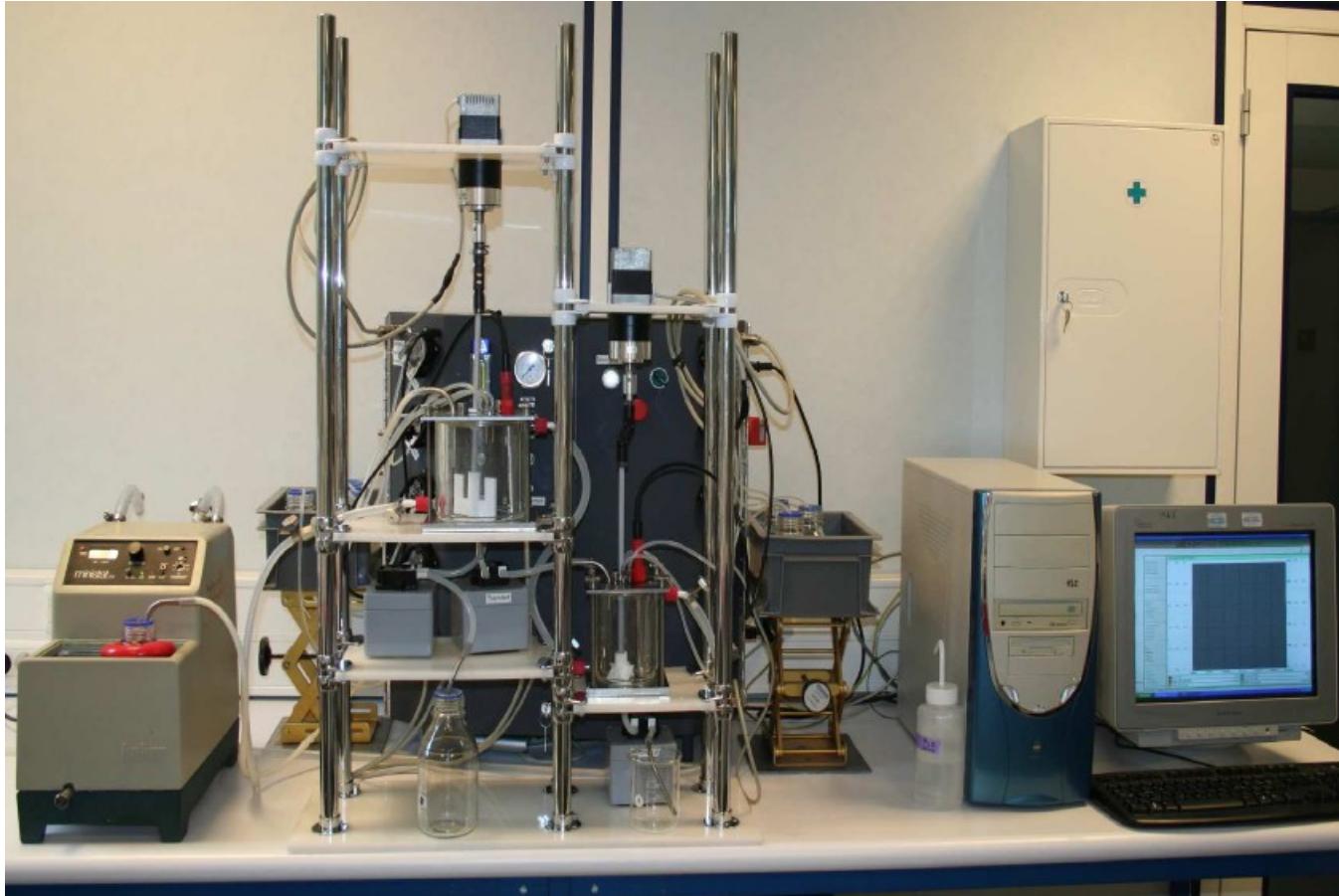
by oscillation :

- determination of the viscoelastic properties of gels formed by acidification of rehydrated milk powders with GDL
- determination of the coagulation time and the firmness of the gel.

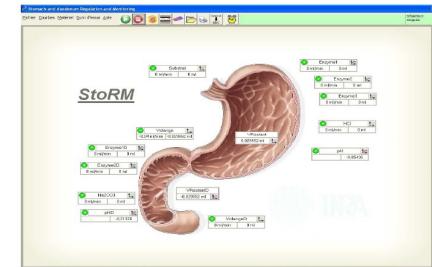


# Development of dynamic digestion models

Cooperation with INRA UMR GMPA (D. Picque *et al.*)

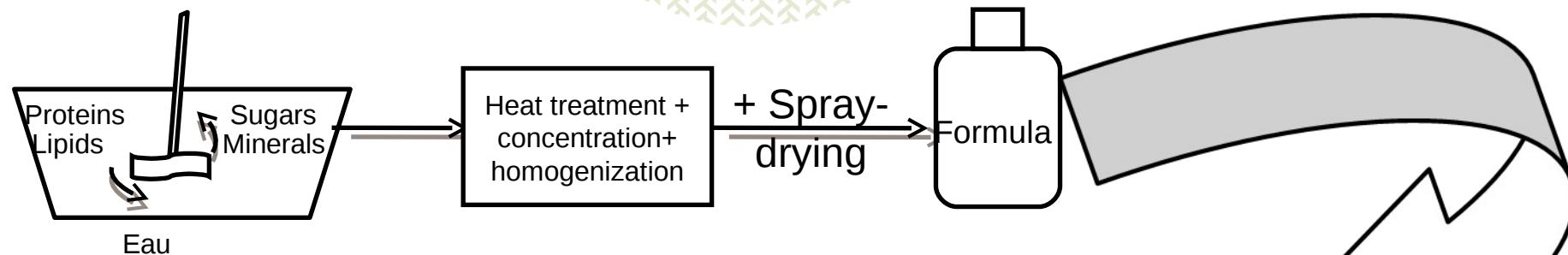


- Logiciel SToRM  
Acquisition des données, stockage, représentations graphique, commandes,...

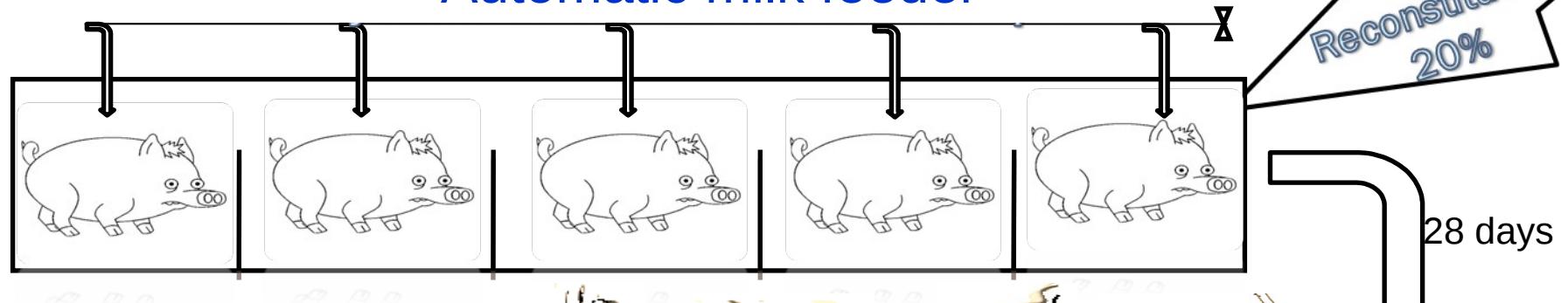


# *In vivo* digestion using the piglet as model

Cooperation with INRA UMR SENAHL (I. Luron et al.)



## Automatic milk feeder



### Effluent Characterization by :

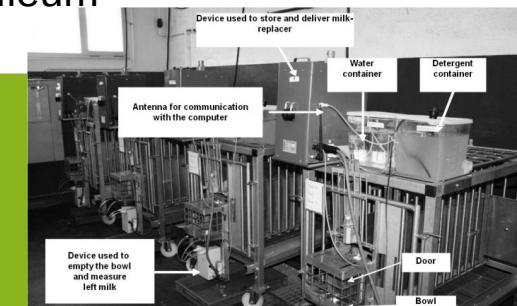
\*SDS-PAGE

\*Immunoassays

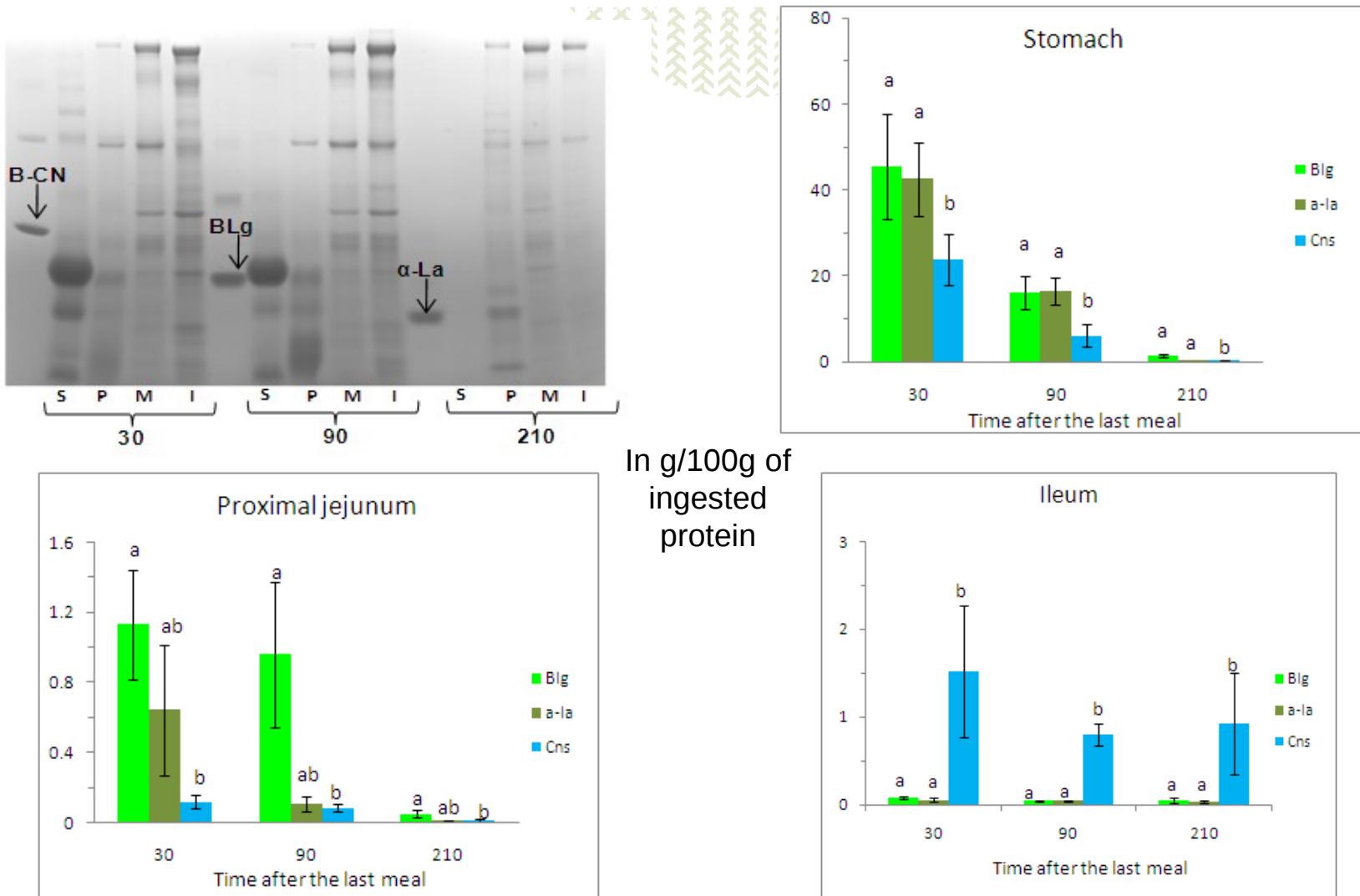
\*LC-MS-MS

Le Croisic 1-3 December

DIET  
AGRICULTURE  
ENVIRONMENT



# Kinetics of Infant Formula proteins digestion



# Peptides identified *in vivo*

For more information, see poster P22

Jejunum at 30 min

**β-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 209  
RELEENIVPGEIVEULUUUEEESITRINKKIEKFOQUEEQOQTEDELODKIHFFAOTOSLVYFFFGRIPNSLQNPPLQTPTVVVPPFLQFPEVMGVSSKVKEAMAAPKKHKEMPPPKYVQPFTEQSLSLTDVNLHFLPLLOSWMHOPHQFLPTVMFPPQSVLSLSQSKVLPVQKA  
VPPQRMPIQAFFLWYQEPVLGPVRGPRPIV

**α<sub>s1</sub>-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 199  
RPKHPRIKHQGLPQEVLNENLLRFPVAPPFPEVGKEKVNELSKNIGUEUTEQAMEDIKQMEAEUUUUEEVPUVQEKHQKEDVPSERLYGLEQLRLKKYVPOLEIPNVAEERLHSMKEGIHAQQKEPMIGVNNELAYFYPELFQRFQYQLDAYPSGA  
WYVPLGTQYTDAPSFSIDPNPIGSENSEKTTMLW

**β-Ig** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160  
UVTOTMKGLDIOKVAGTWYSLAMAASDISLDAQSLAFLRVVVEELKPTPEGDLEILQKWENGECAKKIIAEKTKIPAVFKIDALNENKVVLTDYKKYLLFCMENSAEPEQSLACOCLVRTPEVDDEALEKFDKALKALPMHRLSFNPTQLEEOCHI

**α-la** 1 10 20 30 40 50 60 70 80 90 100 110 120 123  
EQLTKECEVFREKLDKLGYYGVSLFWVCTTFHTSGYDTQAIVQNNNDSTEYGLFQINNKIWCKDDQNPFHSSNCNCSCKFLDDDTDIMCVKKILDKVGINYWLAHKALCSEKLDQWLCEKL

Jejunum at 90 min

**β-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 209  
RELEENIVPGEIVEULUUUEEESITRINKKIEKFOQUEEQOQTEDELODKIHFFAOTOSLVYFFFGRIPNSLQNPPLQTPTVVVPPFLQFPEVMGVSSKVKEAMAAPKKHKEMPPPKYVQPFTEQSLSLTDVNLHFLPLLOSWMHOPHQFLPTVMFPPQSVLSLSQSKVLPVQKA  
VPPQRMPIQAFFLWYQEPVLGPVRGPRPIV

**α<sub>s1</sub>-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 199  
RPKHPRIKHQGLPQEVLNENLLRFPVAPPFPEVGKEKVNELSKNIGUEUTEQAMEDIKQMEAEUUUUEEVPUVQEKHQKEDVPSERLYGLEQLRLKKYVPOLEIPNVAEERLHSMKEGIHAQQKEPMIGVNNELAYFYPELFQRFQYQLDAYPSGA  
WYVPLGTQYTDAPSFSIDPNPIGSENSEKTTMLW

**α<sub>s2</sub>-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 207  
KNTMEHVUUUEEESIIQETYKQENMAINPSKENLCSRKCEVKVRNAEEYSIGUUUEEAEVATEEVKITVODHKYQKALNEINEFYQKFPOYQ/LYQGPVUNPWDQVKRNAVPTPTUNREQLUTEEENSKKTVDMUTEVFTKKTLEEKKRNFLKKISORYQKFALPQYK  
LKTVYQHQKAMKPWIQPKTKVIPYVRYL

**κ-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190  
MMKSFPLVVTIALTLPFLGAQEQNQEQQPIRCEKDERFFSOKIAKYIPIQYVLSRYPSVGUNYYQQKPVAAUNNOFLYPVYYAKPAAVRSFAQJQWQVLNTVPAKSCQIAQPTTMARHPHLFSMAIPKKNQDKTEIPTINTIASGEPTSTPTTEA  
ESTVATLDESPVIESPPEINTVQVTSTAV

**β-Ig** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160  
UVTOTMKGLDIOKVAGTWYSLAMAASDISLDAQSLAFLRVVVEELKPTPEGDLEILQKWENGECAKKIIAEKTKIPAVFKIDALNENKVVLTDYKKYLLFCMENSAEPEQSLACOCLVRTPEVDDEALEKFDKALKALPMHRLSFNPTQLEEOCHI

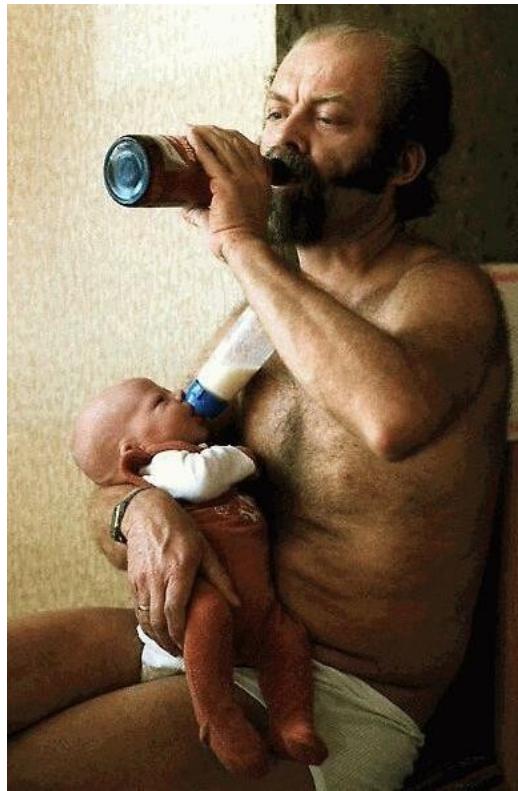
Jejunum at 210min

**β-CN** 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 209  
RELEENIVPGEIVEULUUUEEESITRINKKIEKFOQUEEQOQTEDELODKIHFFAOTOSLVYFFFGRIPNSLQNPPLQTPTVVVPPFLQFPEVMGVSSKVKEAMAAPKKHKEMPPPKYVQPFTEQSLSLTDVNLHFLPLLOSWMHOPHQFLPTVMFPPQSVLSLSQSKVLPVQKA  
VPPQRMPIQAFFLWYQEPVLGPVRGPRPIV

# Conclusion 2

- Caseins are rapidly digested in the stomach compares to whey proteins but caseins fragments are still present in the ileum
- Are these findings compatible with the slow/fast proteins concept of milk???
- One of the key factor might be the ability of the caseins to coagulate in the stomach (depends on their concentration, buffering properties of the matrix...)
- Combination of *in vitro* and *in vivo* approaches are necessary to better understand the mechanisms of milk proteins digestion
- Diversity of analytical techniques allows a sharp characterization of the hydrolysis of proteins in different complexe media (food matrices, effluents...)

# *Improving infant formula for improving human life*



*Thanks for your kind attention !!!*