Ex vivo Ruminal Cellulosome For By-product Biomass Conversion

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RÉGION WALLONNE

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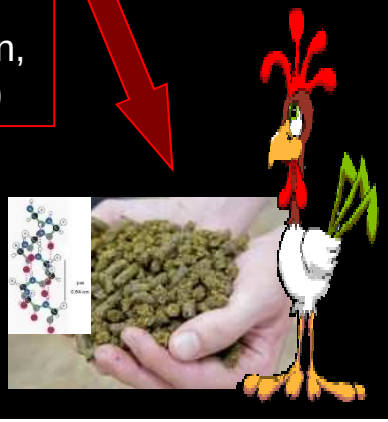
Objective

Production of metabolizable sugars for poultry from biofuel by-products

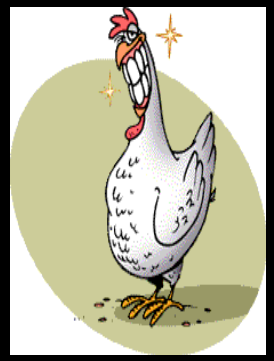
By-products
(wheat distiller grain,
rapeseed meal,…)



Metabolizable

monomers

Chicken



Chicken

- **Energy** : => starch
  => fat
  => ~~cellulose~~

- **Starch future ?** → ↓ Disponibility
  → ↑ Price

- **Solution** = by -products
  ➔ Cellulose + exogen enzymes → glucose

- **HOW ?**
  ➔ Using fibrolytic ruminal enzymes
  ➔ pH and T°of rumen enzymes = pH and T°poultry intest ine

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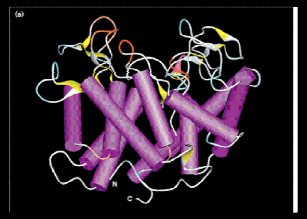
Ruminal fibrolytic enzymes



Agro-alimentary industries

By-products
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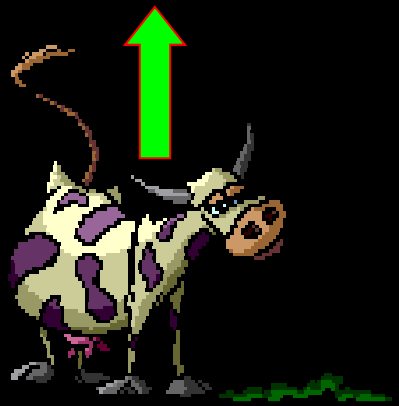
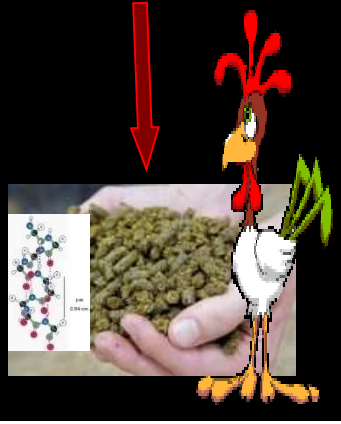




Metabolizable

monomers

Chicken

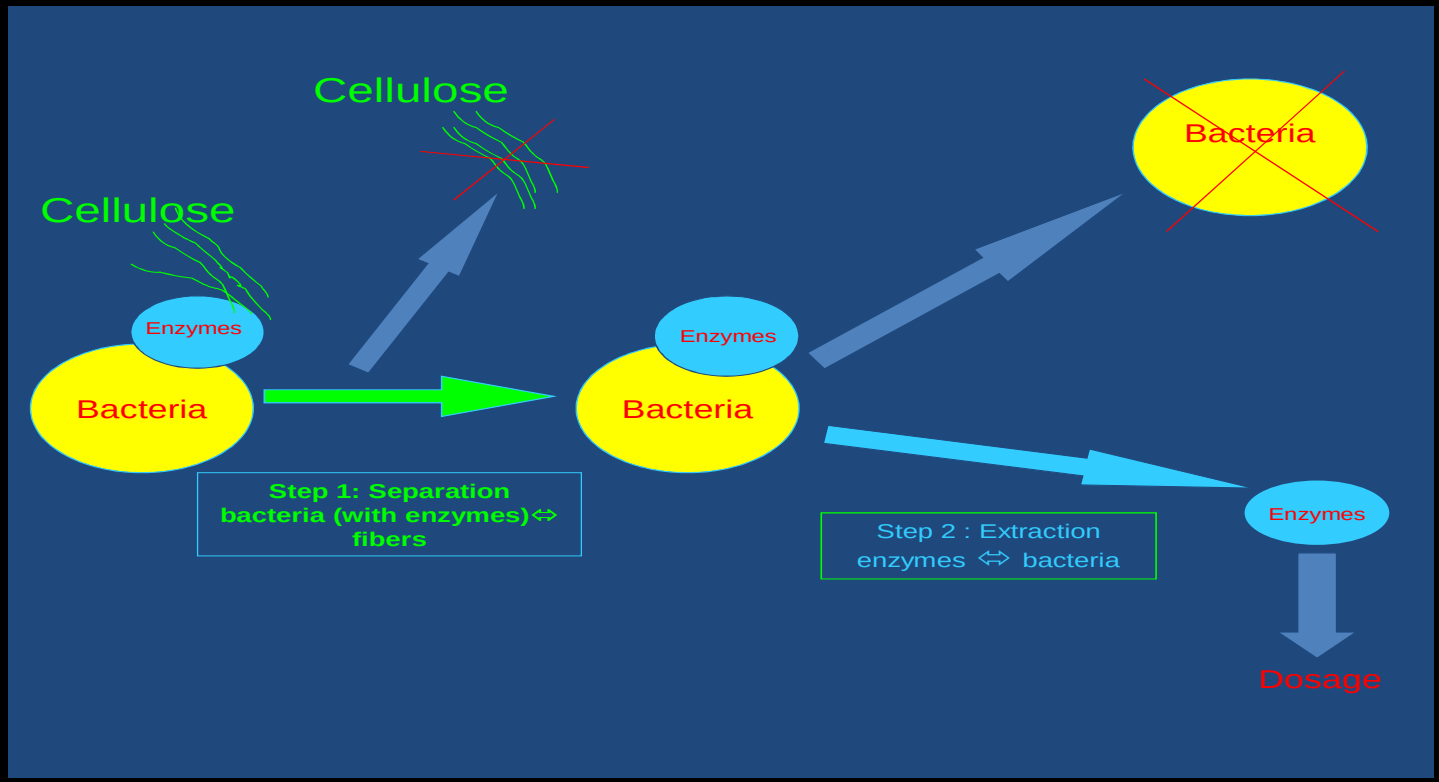
Rumen cellulolytic bacteria

Bacteria associated with feed particles

➔ Up to 75% of the total microbial population in the rumen

➔ Responsible for 88 to 91% of ruminal endoglucanase and xylanase activities

Fibrolytic ruminal crude extract obtention

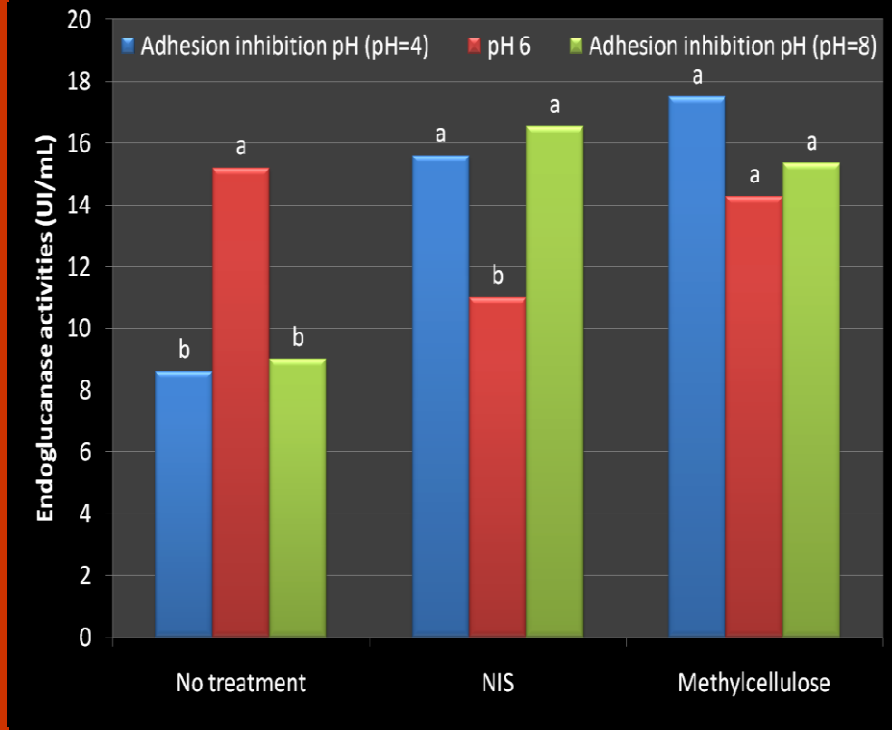


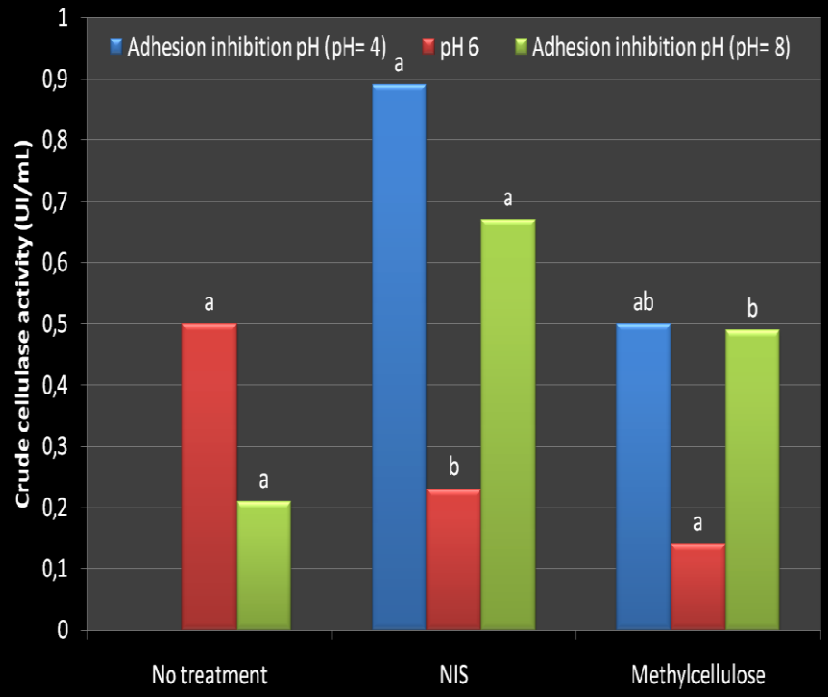
Dosage : Cellulolytic activity measurements
(endoglucanase, exoglucanase, cellobiase and crude cellulase)

Conditions of production

- First step: simulation of adhesion inhibition conditions of bacteria to fibers
- Second step: destabilization of bacteria membran to liberate fibrolytic enzymes

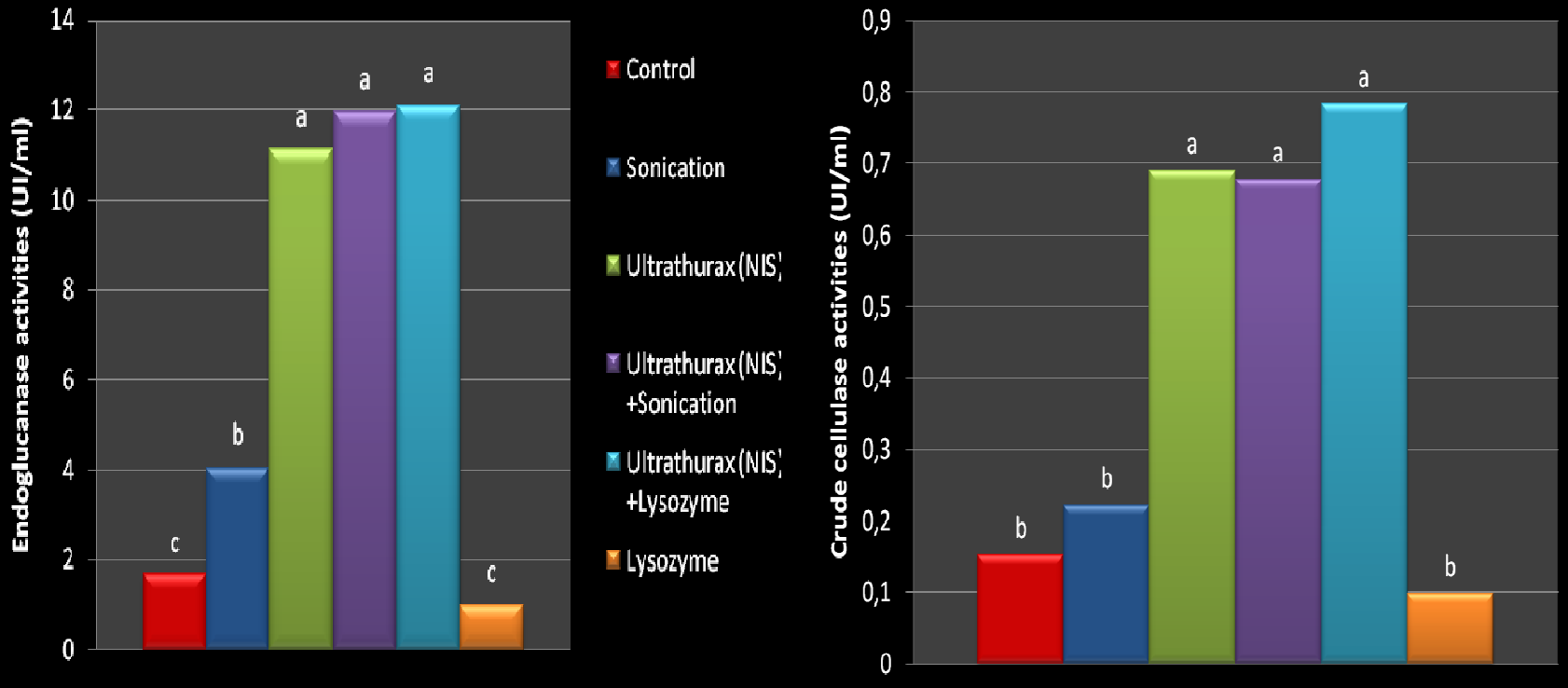
First step
Separation bacteria vs fibers





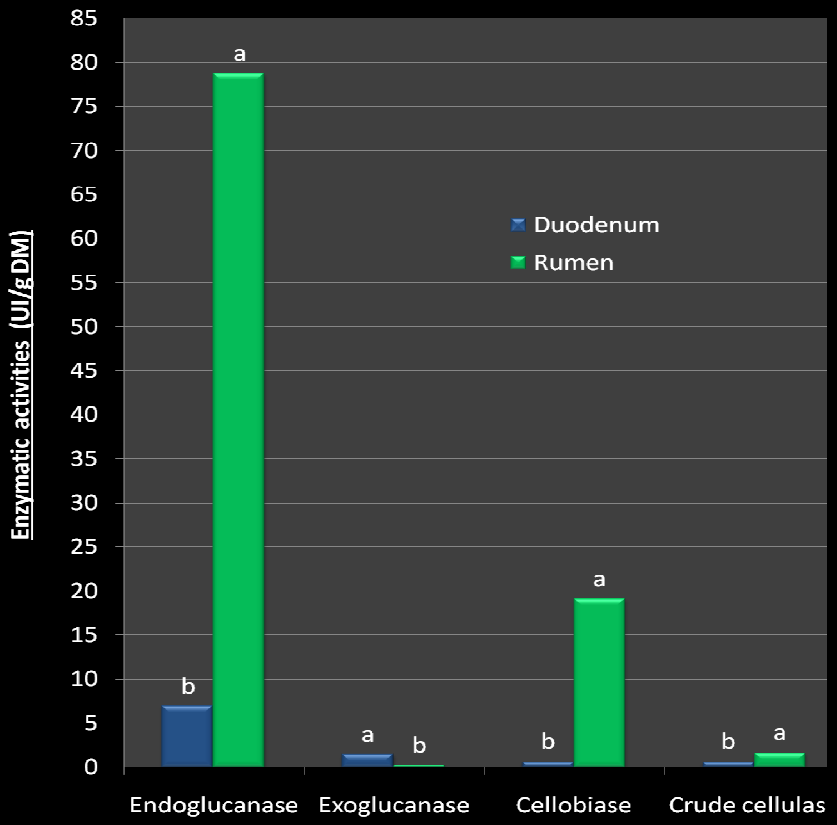
➔ Inhibition adhesion pH in association with NIS/Methylcellulose
=> increase cellulolytic activities

Second step
Extraction enzymes vs bacteria

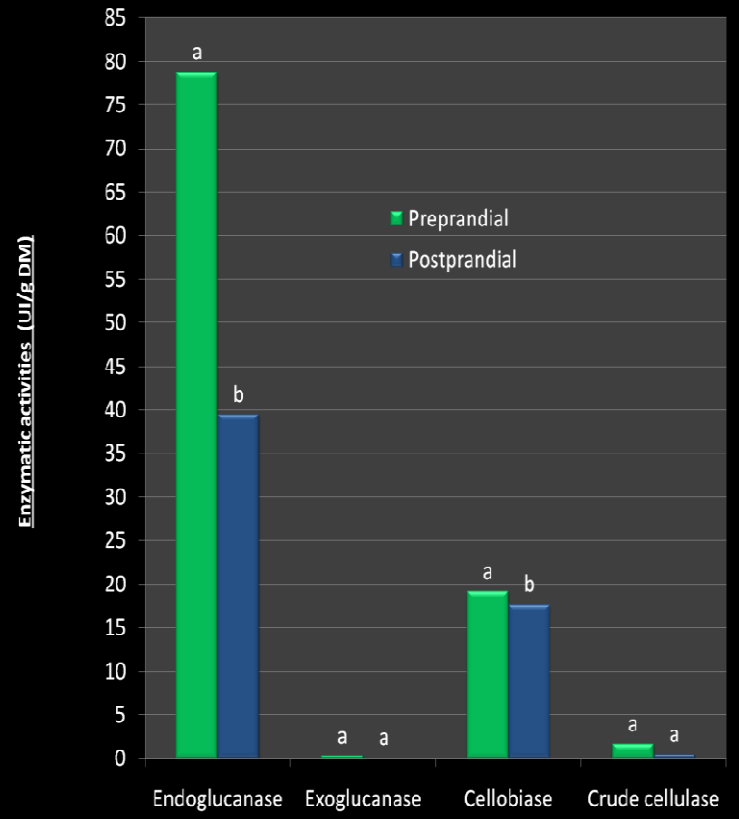


=> Ultrathurax homogenizer (NIS) increase cellulolytic activities

Conditions of extraction
Cellulolytic activitie optimisation

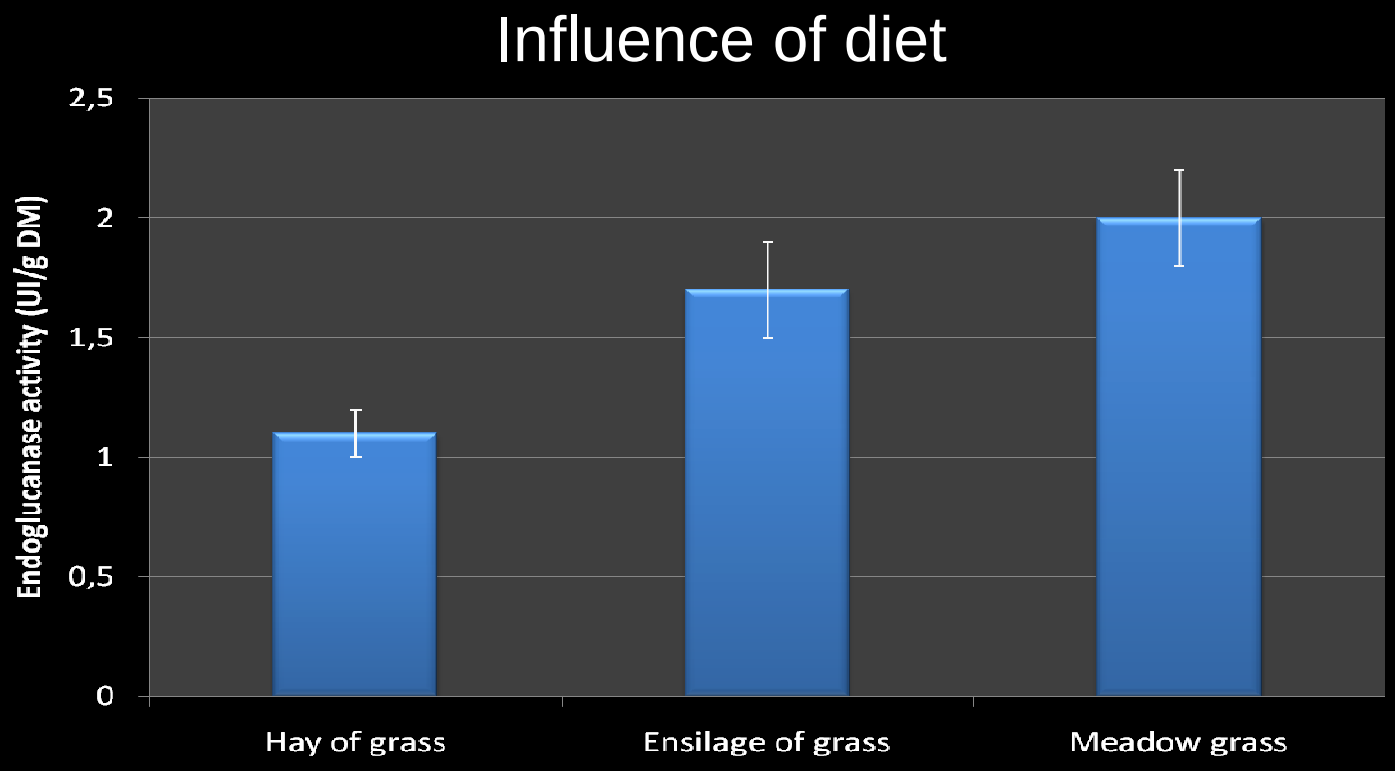


Ruminal activities >>> duodenal activities



Preprandial activities >>>postprandial activities

Conditions of extraction
Cellulolytic activitie optimization



=> Fiber diets enhance cellulolytic activity (meadow grass)

Fibrolytic crude extract definition

Ruminal digesta
(Preprandial – Ensilage grass diet)

↓

Step1: Blended with extracting buffer solution (pH 8 - Methylcellulose)

and homogeneized at 4 ℃

↓

Low speed centrifugation

↓

Step 2: Homogeneisation with Ultrathurax+NIS

↓

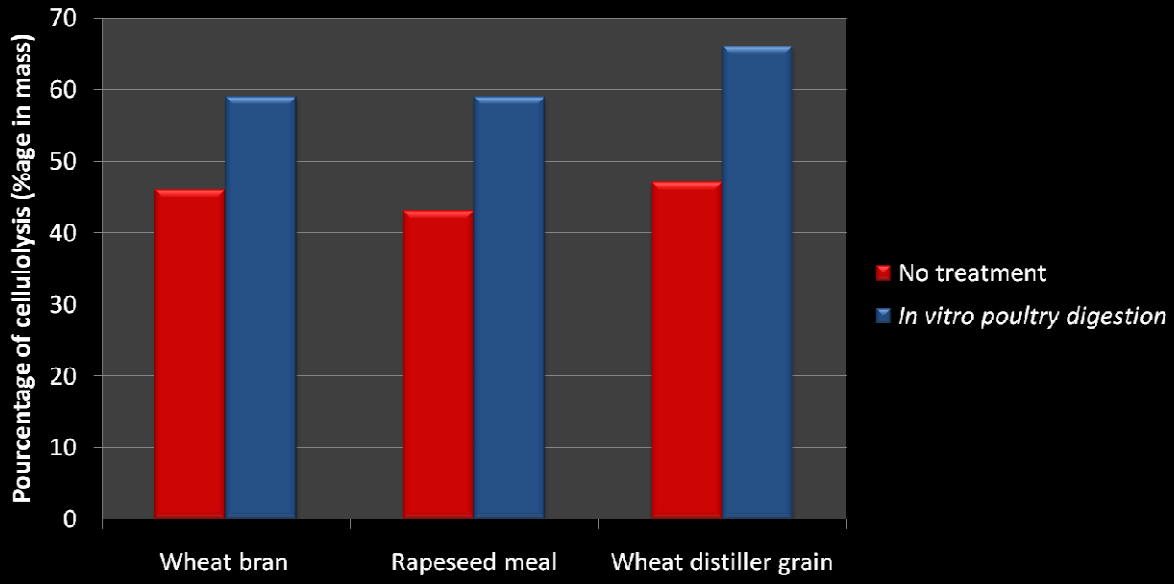
High speed centrifugation

↓

Crude extract solution

By-products cellulolysis by crude extract

Quantity of reducing sugars produced from cellulose in the small intestine with and without *in vitro* digestion simulation (poultry)

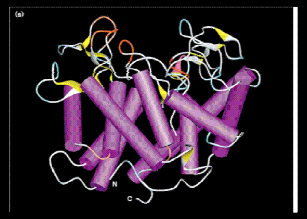


➔Rate of cellulolysis varied from 44% to 66%

Ex vivo production of ruminal fibrolytic enzymes

Production of metabolizable sugars for poultry from biofuel by-products

Ruminal fibrolytic enzymes



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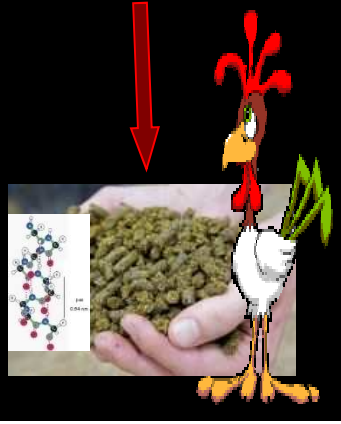

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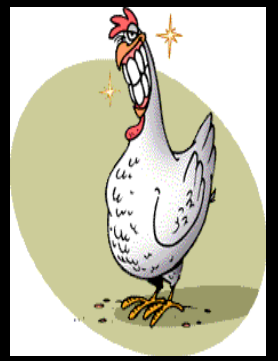

Ex vivo system

- Objective : production of ruminal fibrolytic enzymes from cellulolytic bacteria culture

- 2 types of cultures tested
  - => isolate strain from rumen content
  - => ruminal consortium

Cellulolytic isolate strain

- Identification (BCCM/LMG ; Gent)
  - *Bacillus niabensis*
- Characteristics :
  - Gram +, motile and spore-forming rods
  - Anaerobic and aerobic growth
  - Recently isolate (*Kwon and al.,* 2007) from lignocelluloses (coton, rice straw)

Kinetic of biomass production
Bacillus niabensis

- *Bacillus* medium – aerobic conditions
- Biomass :

  ➔ 10^6 ➡ 10^9 CFU/mL in 96 h
- Sporulation rate up to 60 %
- Enzymatic induction on cellobiose for 24 h

  => 10 UI/ml

Kinetic of biomass production
Ruminal consortium

- Anaerobic condition
- Rumen medium
- pH = 6.8 and T=37℃
- Reducing potential from -200 to -300mV
- Biomass :

➔ 10^7 ➡ 10^9 CFU/mL in 64 h

Production of fibrolytic enzymes in bioreactor (20 L)

- Anaerobic ruminal conditions (on-line regulation)
- Inoculum = anaerobic consortium (10%)
- Substrate = rapeseed meal
- On-line measurement of gaz production
  =>pressor sensor
- Cellulolytic biomass = 10^9 CFU/mL

Conclusions

- Fibrolytic potentialities of ruminal crude extract on biofuel by-products (with *in vitro* digestion)
- Aerobic and anaerobic culture of cellulolytic biomass from ruminal content

Perspectives

- *In vitro*:
  - Optimal rate ruminal cellulases/substrates determination
- *Ex vivo*:
  - Cellulolytic biomass induction
    ➔ ↑ rate enzymes/substrates
- *In vivo*:
  - Performances, digestibilities (HC), metabolisable energies

THANK YOU

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