Free and rumen-protected essential oils incubated *in vitro*: stability and fermentation parameters

Session 48: New technologies to improve feed efficiency

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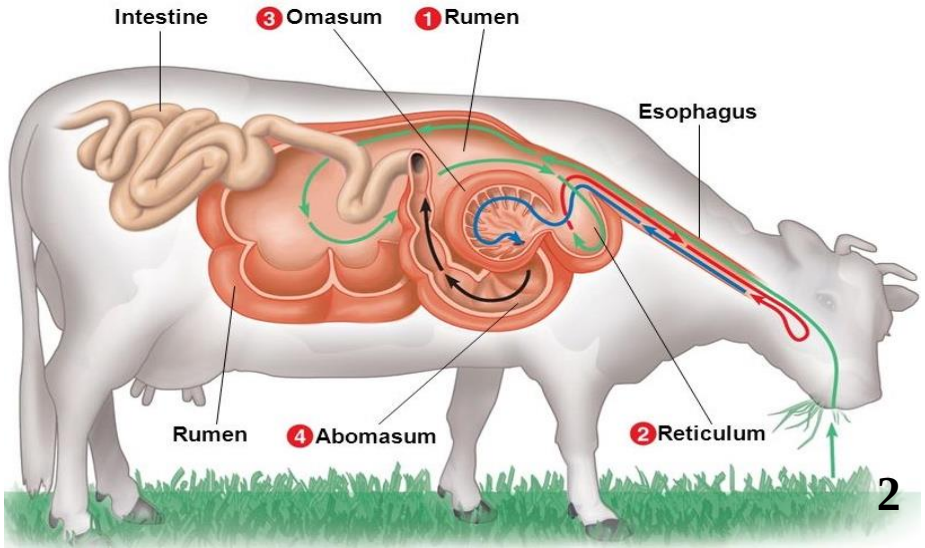
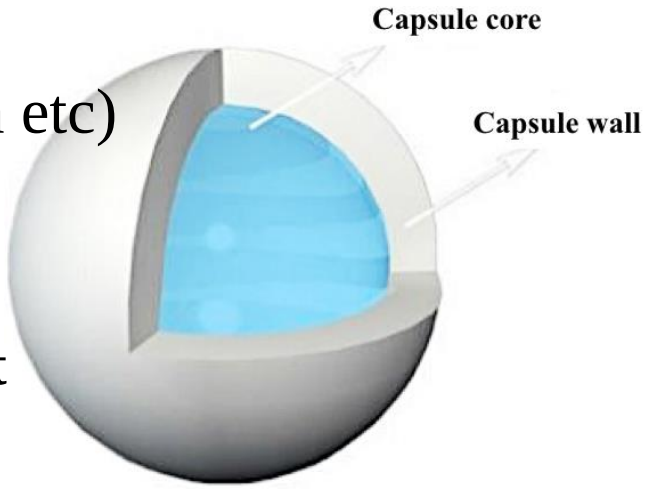
- Antibiotics ban in 2006 resulted in an increased demand of finding alternatives
- **Essential oils (EOs): Cinnamaldehyde, thymol and eugenol** possess antimicrobial properties
- EOs are generally **volatile** that causes hindrance on their usage in animal feed
- Majority of EOs fed to the ruminants get absorbed from rumen
- EOs also get absorbed to feed components



- **Microencapsulation** is an efficient technology that enabled preparation of **stable EO products**

- EOs are coated by a matrix (fatty acids, starch etc)

  - ✓ **Stability to EOs from ruminal degradation**
  - ✓ **Allow site-specific slow release of EO product**



- The proper selection of matrix material to obtain desired degree of stability is often challenging and demand more research

1. Test the **ruminal stability of free and rumen-protected EO** products **in an *in vitro* assay** using **Ankom Daisy^{II} technique**

2. Observe the effects of essential oil products on

   - **rumen pH**
   - **fermentation parameters**
   - **protozoa number**

Tested essential oil products

3 essential oils in 2 different form

- Free (fEOs)
- Microencapsulated/rumen-protected (rpEOs)

Olistat-G

Olistat-P

Olistat-Cyn 20%

rpEOs	Olistat-G	Olistat-P	Olistat-Cyn 20%
Components	1. Cinnamaldehyde (3%) 2. Vitamins 3. Pro-vitamins	1. Cinnamaldehyde 2. Thymol 3. Eugenol	1. Cinnamaldehyde (20%)
Protection matrix	• Vegetable hydrogenated fatty acids • $CaCO_3$ • **Corn starch** • **Vegetable extracts**	• Vegetable hydrogenated fatty acids • $CaCO_3$	• Vegetable hydrogenated fatty acids • $CaCO_3$ • **wheat flour**

Working principle of Ankom Daisy^{II} technique

1. Relies on filter bag technology, which encapsulate samples and prevent filtration errors

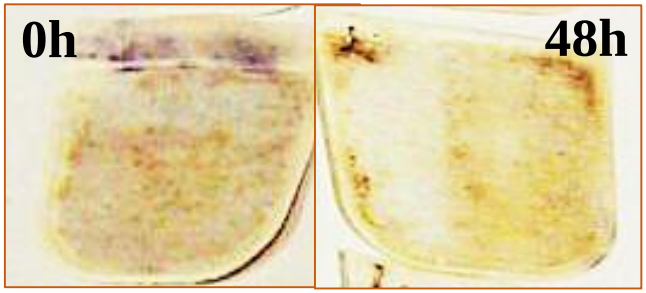
2. Allow simultaneous incubation of different additives in same vessel

25 µm pore size

2. Comprises of four digestion vessels

   - **4L capacity each**
   - **rotate continuously to allow mixing of inoculum**

3. Material that disappears from the bag is considered soluble and digestible



4. Ruminal stability of products was calculated by recording dry matter disappearance (DMD)

Experimental Setup

15 g
Dry cows diet

1600 ml
Buffer

400 ml
rumen fluid

0.2-1.0 g
EO Product

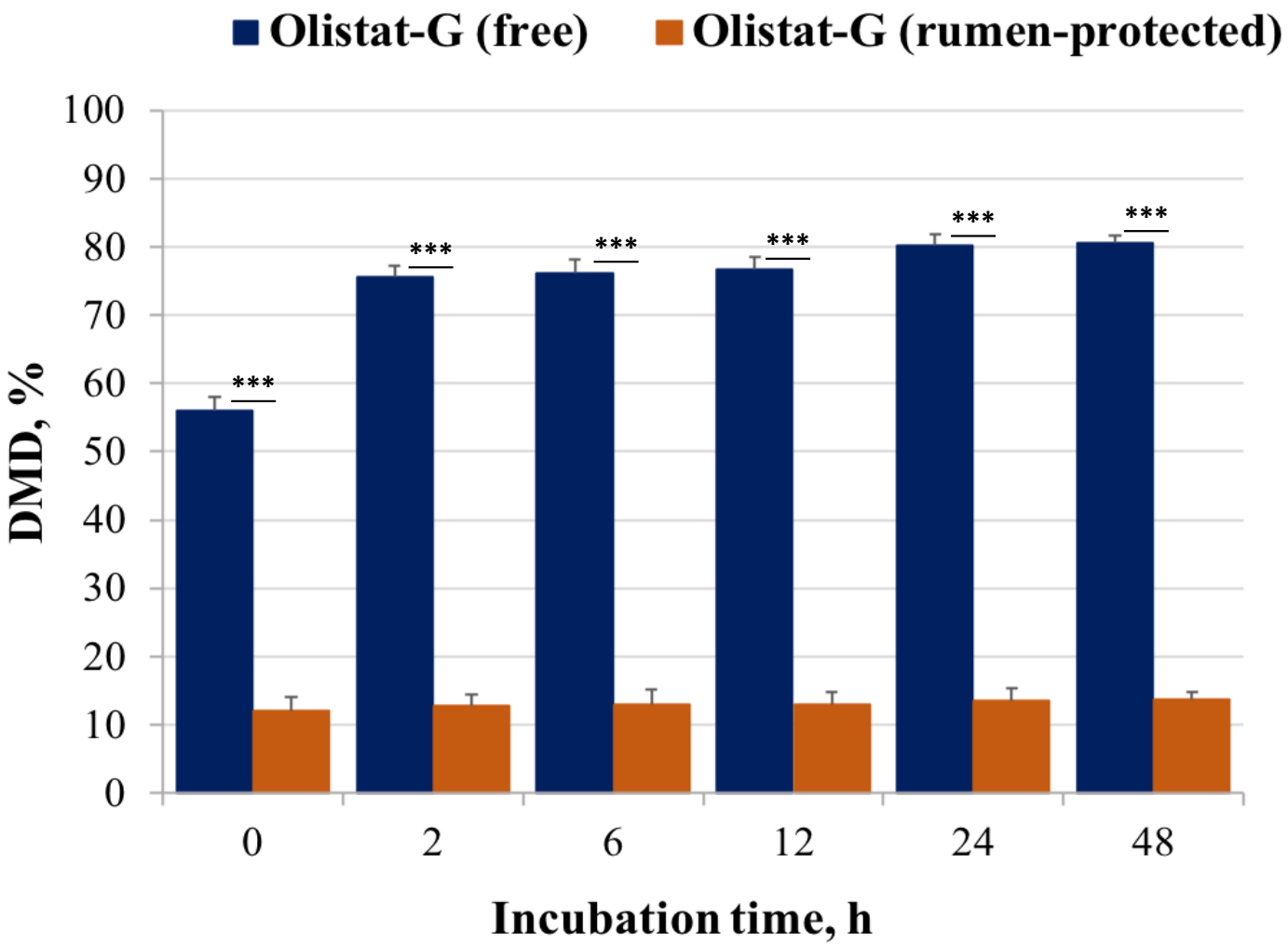
Free EO & rumen-protected EOs in same jar

(Menke and Steingass, 1988)

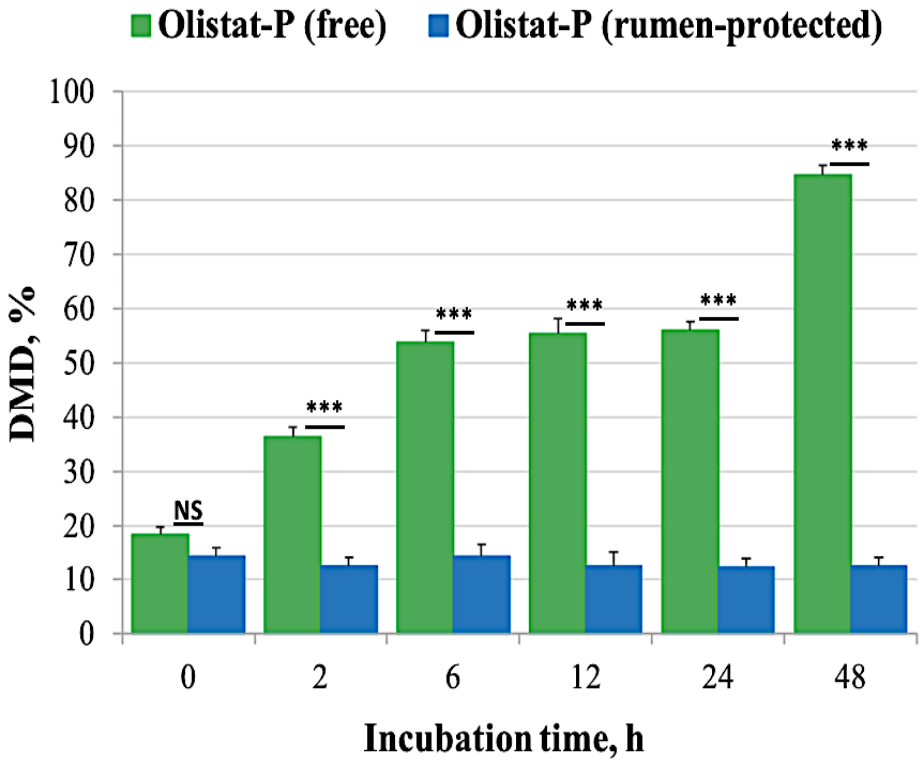
Dry matter disappearance (DMD) of EOs
(0, 2, 6, 12, 24, and 48 h)

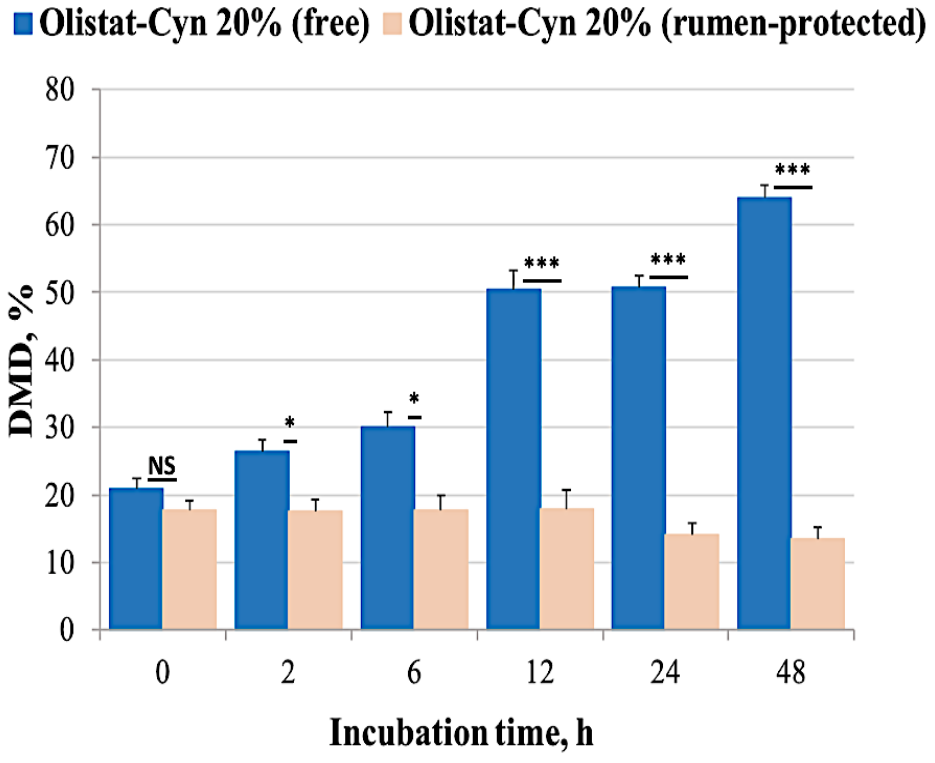
Fermentation parameters and protozoa No. → **(0 and 48 h)**

1. Ruminal stability of essential oil product: OLISTAT-G



2. Ruminal stability of essential oil products: OLISTAT-P &OLISTAT-Cyn 20%





3. Rumen fermentation parameters and protozoa number of EOs products

Product	No additive	Olistat-G (f+rpEO)	Olistat-P (f+rpEO)	Olistat-Cyn (f+rpEO)	RMSE	*P*-value
Incubation time (h)	**0**	48	48	48		
pH	**6.93^{B}**	6.36^{A}	6.46^{A}	6.73^{AB}	0.12	<0.001
Protozoa (log10/mL)	**4.73^{b}**	4.40^{a}	4.56^{ab}	4.72^{ab}	0.19	0.099
Total-VFA (mmol/L)	**17.0^{a}**	33.0^{b}	27.0^{ab}	18.0^{ab}	9.8	0.095
VFA (%)						
Acetate	**71.7**	71.6	72.4	70.4	4.5	0.96
Propionate	**16.6**	18.0	16.1	18.2	3.5	0.82
Butyrate	**8.4**	7.9	9.7	9.1	1.8	0.61
Acetate : Propionate	**4.31**	4.67	4.96	3.88	1.0	0.71

Legends - RMSE: root mean square error

^{a,b,c} different superscripts within a row indicate, means differ ($P \leq 0.05$)

^{A,B,C}different superscripts within a row indicate, means differ ($P \leq 0.001$)

Stability

Free essential oils (fEOs)

- rapidly degraded
- with maximum disappearance of products observed at 48 h of incubation

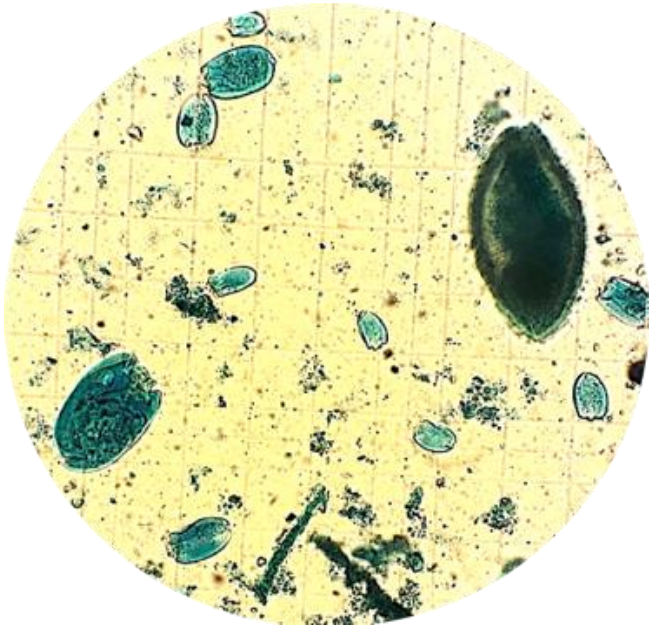
Microencapsulated/rumen-protected essential oils (rpEOs)

- **Highly stable** with relatively **low disappearance in the ruminal environment**

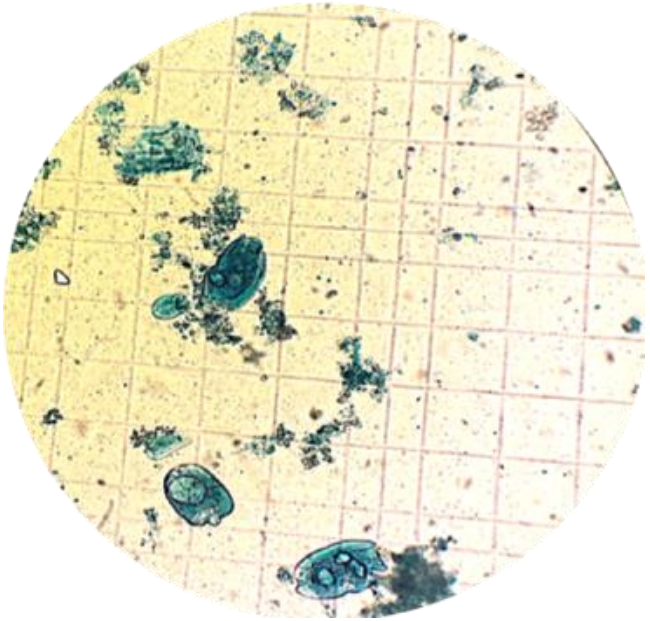
Form of products	Free (fEO)	Rumen-protected (rpEO)
Olistat-Cyn 20%	64.0%	13.4%
Olistat-G	80.5%	13.7%
Olistat-P	84.6%	12.4%

Fermentation parameters & protozoa number

- **Only OLISTAT-G** caused a significant **effect on fermentation parameters & protozoa No.**



At 0h of incubation



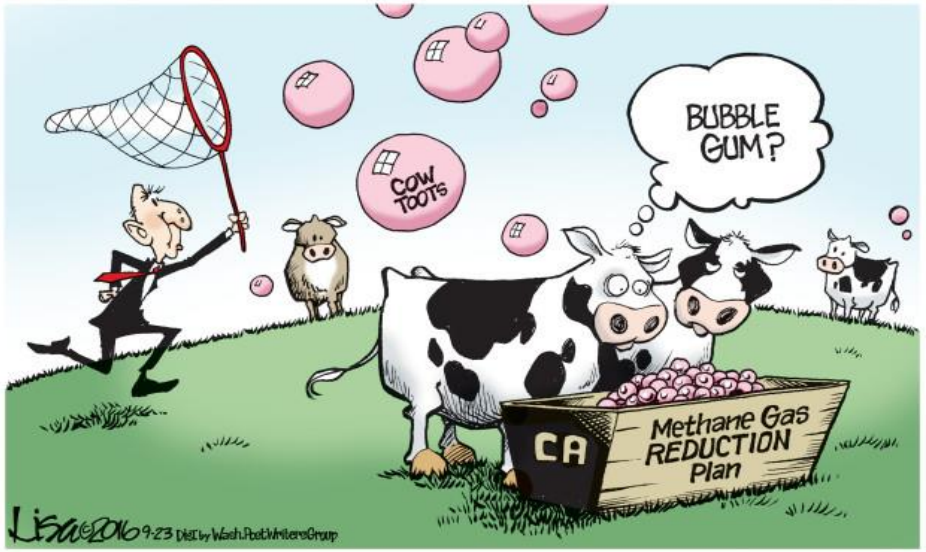
At 48h of incubation

1. The protection of EOs from ruminal degradation by microencapsulation was found to be very effective to ensure the rumen by-pass.

2. Olistat-G was capable of changing rumen fermentation

Future perspective

In vivo experiments can be conducted in future using Olistat-G, to verify the dosage and long-term beneficial effects of this EO in ruminants



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THANK YOU FOR YOUR ATTENTION

- Statistical analysis was conducted using PROC GLM procedure in SAS
- Data of dry matter disappearance (DMD, % DM) was analyzed considering in the model

✓ **the effect of EO products (6 levels: three EOs in two different forms)**

✓ **the effect of incubation time (6 levels: 0, 2, 6, 12, 24, and 48 h) and**

✓ **the interaction EO products x incubation time**

```
proc mixed data=SILA_FINALic;
class Time EO products;
model Corrected_DMD =EO products|Time/DDFM=KR OUTP=R;
repeated / group=Time;
lsmeans Time*EO products / diff adjust=Tukey;
run;
quit;
```

```
data SILA_FINAL_t0;
set SILA_FINAL1;
if Time='0';

proc glm data=SILA_FINAL outstat=ANOVA_NIDA0;
class EO products;
model Corrected_DMD = EO products;
*test h=metodo e=incubaz(metodo);
*test h=metodo*alimento e=incubaz(metodo);
*test h=metodo*alimento*incubaz(metodo) e=incubaz(metodo);
lsmeans EO products / stderr out=LSMEANS_NIDA0;
contrast Olistat-G_F vs Olistat-G_protected' Additive  1 -1  0  0  0  0;
contrast Olistat-P_F vs Olistat-P_protected' Additive   0  0  1 -1  0  0;
contrast Olistat-G_F vs Olistat-G_protected' Additive  0  0  0  0  1 -1;
run;
quit;
```