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Evolution of the rumen fluid enzymatic activity during *in vitro* incubation

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INTRODUCTION – 1 - Background

- Fresh rumen fluid (RF) as «degrading agent» (Goering and Van Soest, 1970)

- **Digestibility**: *In vitro* techniques (Goering and Van Soest, 1970)

relatively **cheap** and **quick**, sometimes lack in repeatability

17% inter- and intra-laboratories variability of the NDF digestibility values at 30 h of fermentation (Hall and Mertens, 2012)

RF: Affected by individual and dietary factors (Chaundhry, 2008)

INTRODUCTION – 2 - Background

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INOCULUM

- pooled from several **donor cows** - legal constrains (Directive 2010/63/UE)
- obtained from **slaughtered animals** - variability - culled cows? (Chaudhry, 2008)
- **priming** - improved repeatability but depressed fiber digestibility (Goeser and Combs, 2009)

INTRODUCTION – 3 – Hypothesis and aim

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ASSUMPTION

Rumen fluid (RF) enzymatic activity: expression of its degradative potential

HYPOTHESIS

The *in vitro* incubation of different RFs on a common priming complete substrate can reduce the differences between their degradative potential

AIMS

Describe the evolution of enzymatic activities of different RFs during a priming *in vitro* incubation.

Determine the priming fermentation **interval minimizing the differences** between RFs enzymatic activities.

MATERIALS AND METHODS - 1

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RUMEN FLUIDS

Rumen fluids (RFs) collected from

Dry cows (D)	Lactating cows (L)
100% **hay** **DTH** diets	**60:40** F:C **hay** + **concentrate separately** **LFC** diets
80:20 F:C **forage**:**concentrate separately** **DFC** diets	60:40 F:C **total mixed rations** **LTMR** diets

3 farms/diet, 3 donor cows/farm.

RFs were pooled by farm, divided in 2 flasks
Inoculated at a ratio of 1:4 with medium and 5 g of a **common substrate as a primer** –a P**TMR diet**-

Chemical composition of the rumen fluids derived diets and of the priming diet (PTMR)

	DTH[a]			DFC			LFC			LTMR			PTMR[b]
Item	mean	±	SD	mean	±	SD	mean	±	SD	mean	±	SD	Amount
Dry matter (DM) (%, as fed)	**84.98**	±	2.90	**85.84**	±	3.02	**85.98**	±	0.27	**73.73**	±	7.24	**63.4**
						% DM							
Ash	**8.71**	±	0.84	**9.30**	±	0.77	**8.63**	±	0.50	**7.37**	±	0.72	**8.61**
Crude protein	**9.93**	±	1.22	**9.86**	±	0.59	**15.23**	±	2.09	**16.08**	±	0.54	**16.67**
Ether extract	**1.56**	±	0.07	**1.77**	±	0.04	**3.65**	±	1.13	**2.43**	±	0.74	**3.23**
Starch		-		**8.14**	±	1.62	**17.14**	±	3.82	**21.77**	±	3.44	**22.77**
Non fibre carbohydrates (NFC)	**18.56**	±	3.33	**23.85**	±	1.35	**32.42**	±	3.51	**38.75**	±	4.24	**34.27**
aNDF	**61.23**	±	2.20	**55.23**	±	2.05	**40.08**	±	6.10	**35.37**	±	3.19	**37.22**
ADF	**39.74**	±	4.52	**32.42**	±	1.31	**25.25**	±	3.64	**20.26**	±	3.81	**20.28**
Lignin (sa)	**7.93**	±	2.78	**4.10**	±	1.00	**4.07**	±	0.49	**4.35**	±	0.16	**3.55**
Hemicellulose	**21.49**	±	3.17	**22.81**	±	0.92	**14.83**	±	2.64	**15.11**	±	0.85	**16.94**
Cellulose	**31.81**	±	2.10	**28.32**	±	0.58	**21.18**	±	3.16	**15.91**	±	3.84	**16.73**
NDFD24	**33.10**	±	6.09	**54.70**	±	0.91	**47.15**	±	4.49	**48.01**	±	1.96	**70.83**
F:C	**100:0**			**80:20**			**60:40**			**60:40**			**40:60**

MATERIALS AND METHODS - 2

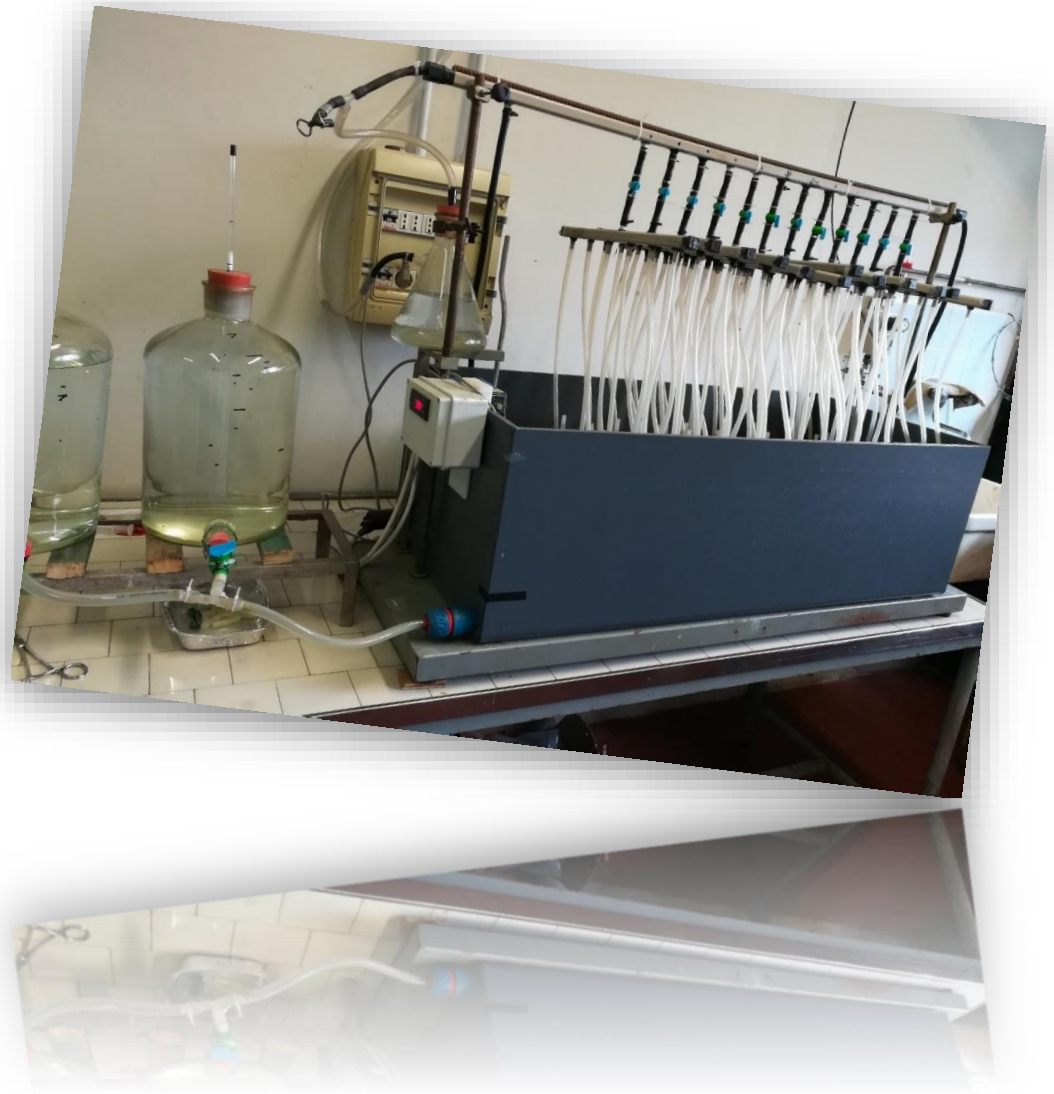
INCUBATION

“in vitro” batch fermentation system

Incubation: 39.5 °C, anaerobic conditions, 48 hours

RFs sampling: duplicate at **0, 1, 2, 4, 8, 24 and 48** h of incubation

Samples were centrifuged and supernatanats were filtered through PVDF 0.45 µm porosity filters for the enzyme activity determination



MATERIALS AND METHODS - 3

ENZYMATIC ACTIVITY EVALUATION:

Radial enzyme diffusion method (RED) – Walsh et al. (2005)

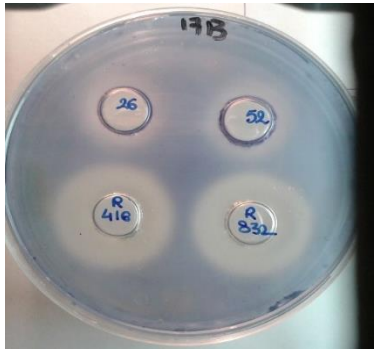
Petri dishes were poured with a gel containing a **specific substrate and buffer**
4 wells/dish
300 µl of rumen fluid/weel

Time of incubation: 16 h

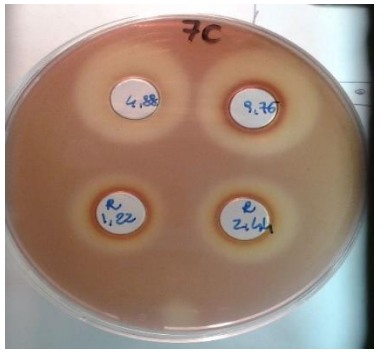
Temperatures: 50°C for **cellulase** (Cell) and **amylase** (Amy)
37°C for **xylanase** (Xyl)

Hydrolysis halos: were revealed by **staining plates with Lugol** solution at different concentrations **Cell** and **Amy**
Hydrolysis halos of **Xyl revealed directly** after incubation

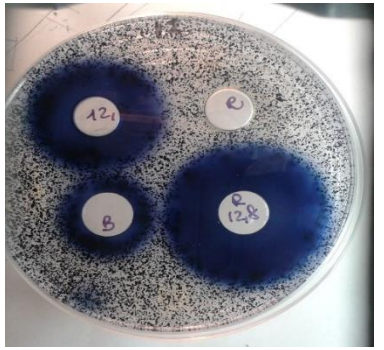
Amylase



Cellulase



Xylanase



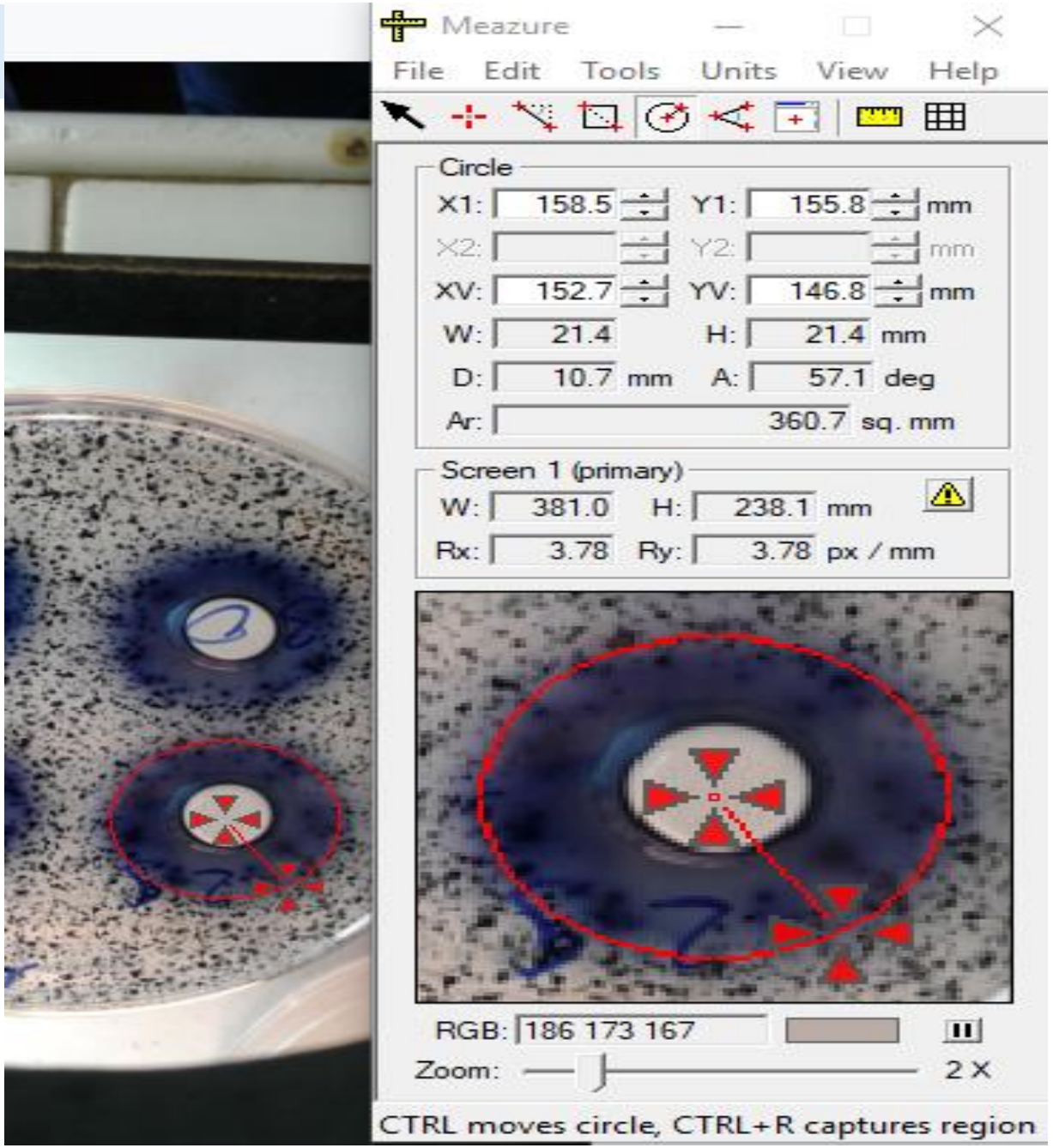
MATERIALS AND METHODS - 4

ENZYMATIC ACTIVITY MEASUREMENT

Halos dimension: measured with **Meazure software®** and EA was expressed as **area of the surface of the halos**.

STATISTICAL ANALYSIS

Repeated measures procedure of the general linear model using **DT as a fixed factor**, farm as random effect and intervals as repeated measures.



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RESULTS AND DISCUSSION - 1

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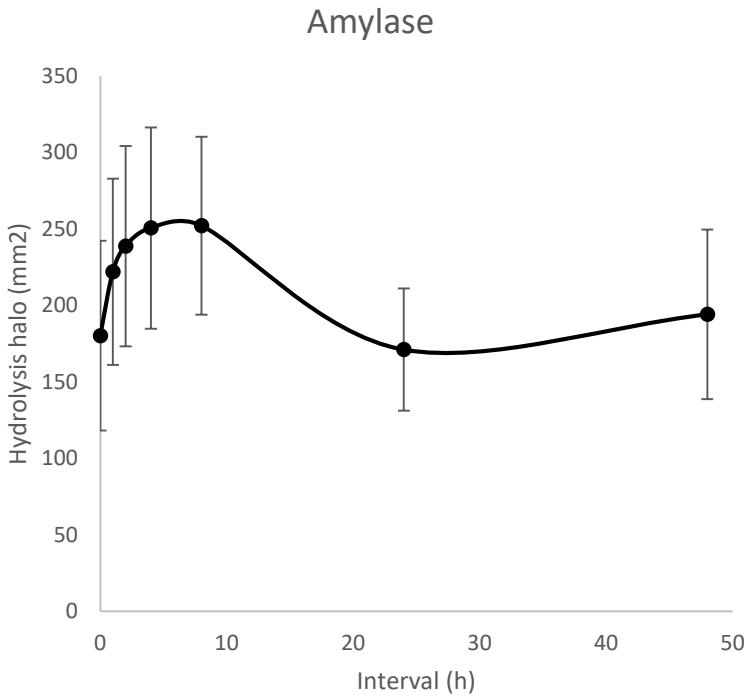
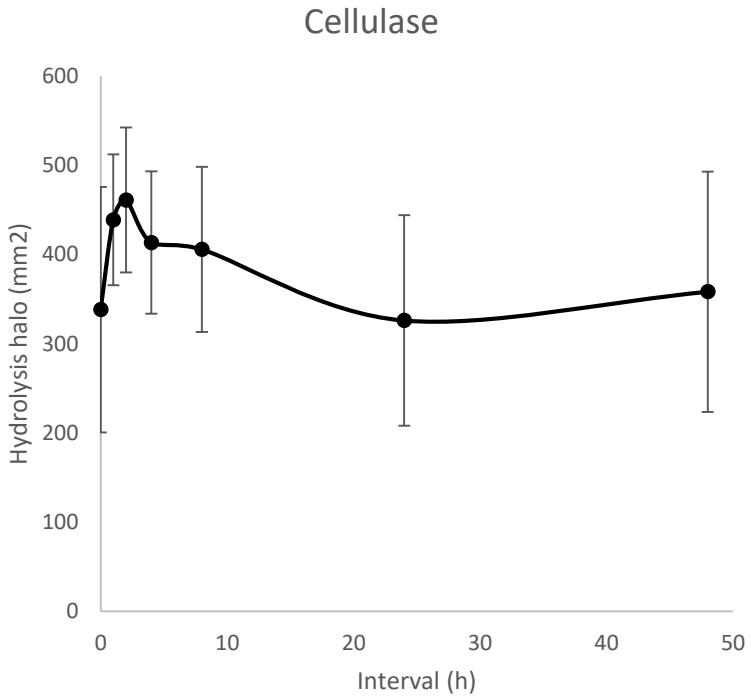
Variable initial EAs, similar by physiological phase for Amy and Xyl; initial Cell levels growing from TH to TMR

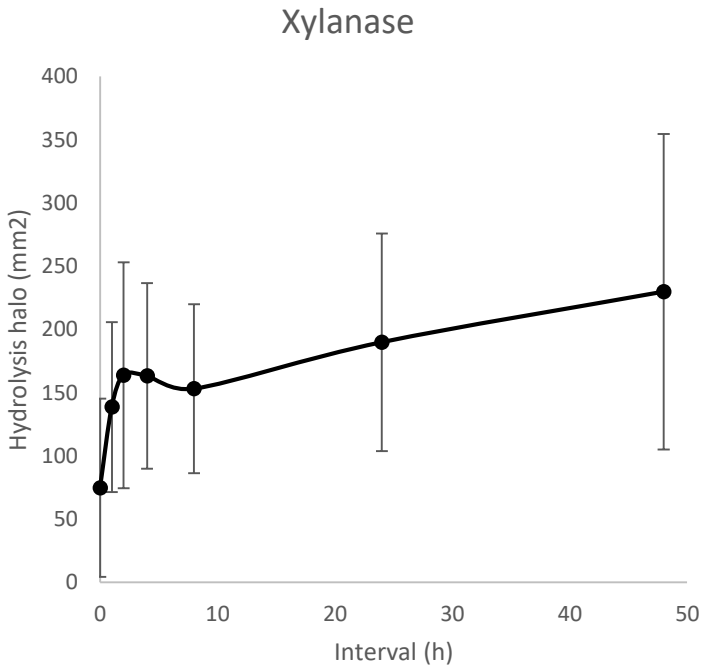
	INTERVAL																				P		
AMILASE	0			1			2			4			8			24			48			SEM	Diet	Interval	D*I
TH	**135,61**	a	**A**	197,81	b	B	220,65	c	B	231,88	cd	A	260,13	e	B	200,03	b	C	234,38	d	D	3,236			
FC	**135,84**	a	**A**	180,91	c	A	183,75	c	A	216,99	d	A	233,37	e	A	141,54	a	A	153,15	b	A	3,203	<0,001	<0,001	<0,001
LFC	**225,93**	c	**B**	265,25	d	D	280,86	ef	C	284,74	f	C	272,12	de	B	171,65	a	B	186,63	b	B	3,527			
TMR	**223,40**	c	**B**	244,21	d	C	269,51	e	C	268,55	e	B	242,63	d	A	171,20	a	B	202,42	b	C	3,542			
SEM	4,478			4,393			4,726			4,747			4,193			2,885			3,997						
P	<0,001			<0,001			<0,001			<0,001			<0,001			<0,001			<0,001						
CELLULASE																									
TH	**202,79**	a	**A**	356,85	b	A	397,45	c	A	352,34	b	A	353,05	b	A	415,26	c	B	504,15	d	C	7,767			
FC	**278,33**	a	**B**	423,87	de	B	415,96	d	B	388,59	c	B	437,18	e	B	291,19	a	A	317,13	b	B	4,639			
LFC	**371,42**	d	**C**	476,51	f	C	479,53	f	C	403,13	e	B	350,46	c	A	290,91	a	A	325,31	b	B	5,031	<0,001	<0,001	<0,001
TMR	**499,92**	cd	**D**	497,94	cd	D	550,85	e	D	509,27	d	C	481,88	c	C	306,67	b	A	286,23	a	A	6,246			
SEM	9,917			5,301			5,866			5,760			6,665			8,519			9,716						
P	<0,001			<0,001			<0,001			<0,001			<0,001			<0,001			<0,001						
XILANASE																									
TH	**44,71**	a	**A**	116,41	b	AB	100,24	b	A	123,48	b	A	119,42	b	A	254,15	c	C	338,86	d	B	11,974			
FC	**42,60**	a	**A**	115,44	b	AB	176,52	cd	B	125,78	b	A	158,43	c	B	140,56	bc	A	191,56	d	A	7,016			
LFC	**91,29**	a	**B**	173,37	bc	C	220,19	d	C	198,02	cd	B	143,63	b	AB	188,14	cd	B	197,77	cd	A	5,698	<0,001	<0,001	<0,001
TMR	**120,54**	a	**C**	149,44	b	BC	158,29	b	B	205,39	cd	B	190,73	c	C	176,37	bc	AB	190,85	bc	A	7,493			
SEM	8,302			7,914			10,518			8,640			7,864			10,140			14,686						
P	<0,001			0,002			<0,001			<0,001			<0,001			<0,001			<0,001						

RESULTS AND DISCUSSION - 2

After an initial irregular peak of activity (2-4 h), a gradual reduction of EA was observed toward the 24 h interval, with increased values at 48 hours (exception: Xyl)







RFs derived from TH showed a different evolution for both Cell and Xyl, with growing values starting from the 8 hrs interval

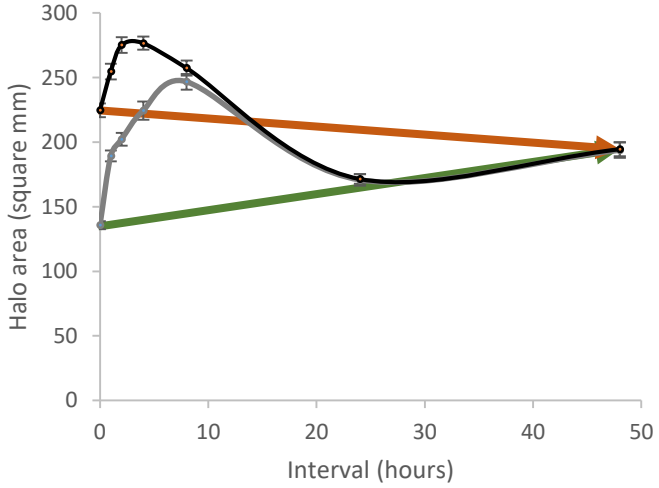
RESULTS AND DISCUSSION - 3

The RFs derived from high fiber diets improved their EAs at 24 and 48 h of fermentation

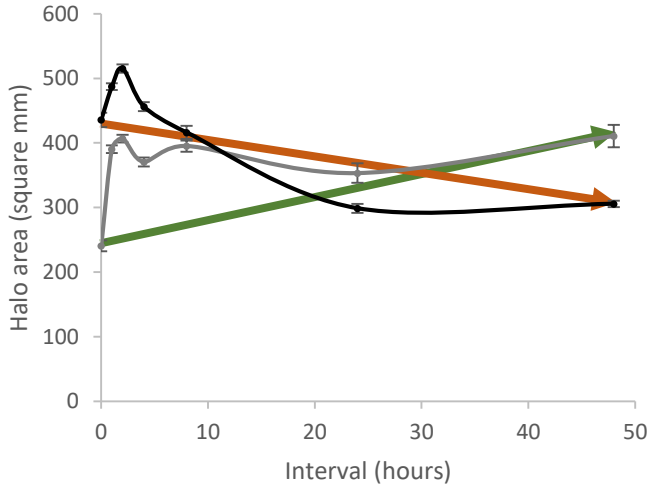
The RFs derived from low fiber diets showed final levels of EAs lower than the initial ones

Xyl was improved by the priming process in all cases

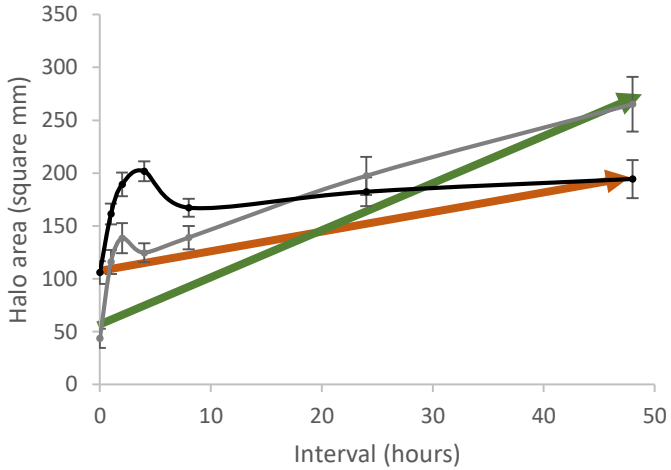
Amylase



Cellulase

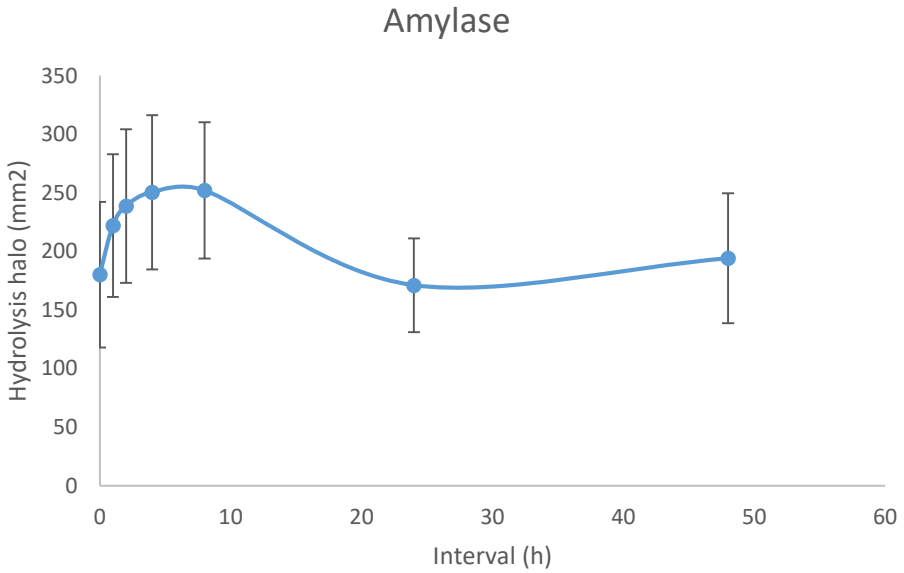


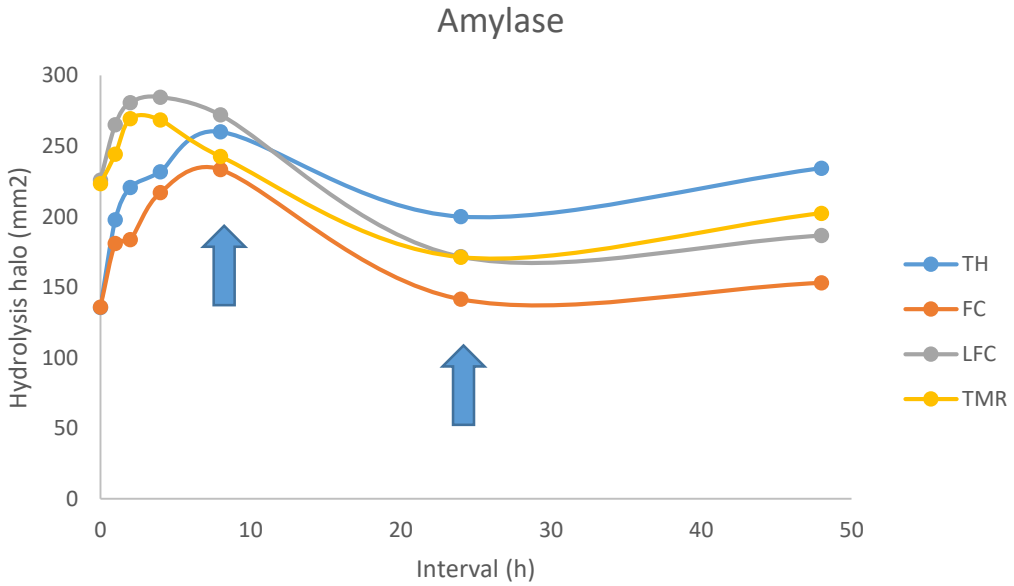
Xylanase



High concentrate Low concentrate

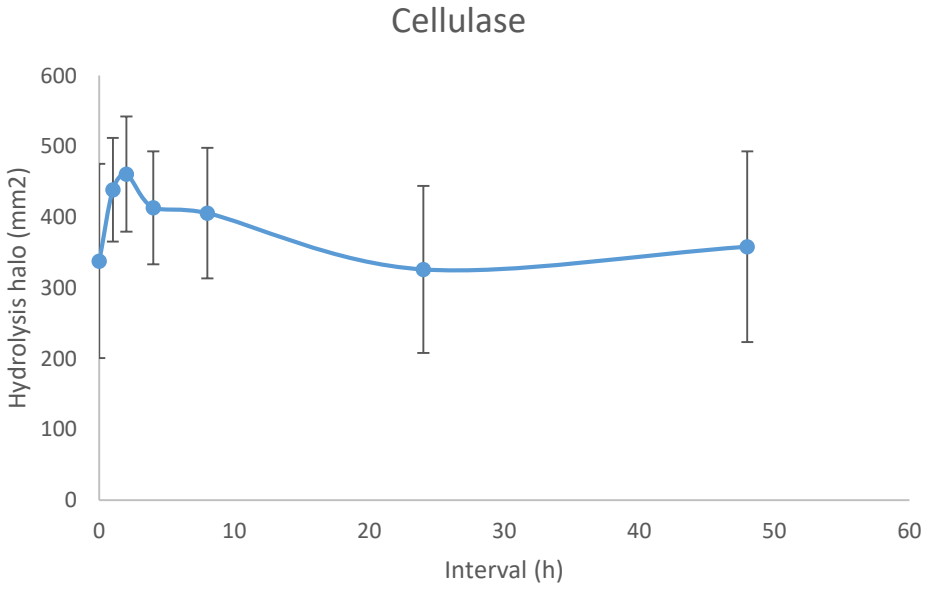
RESULTS AND DISCUSSION - 4

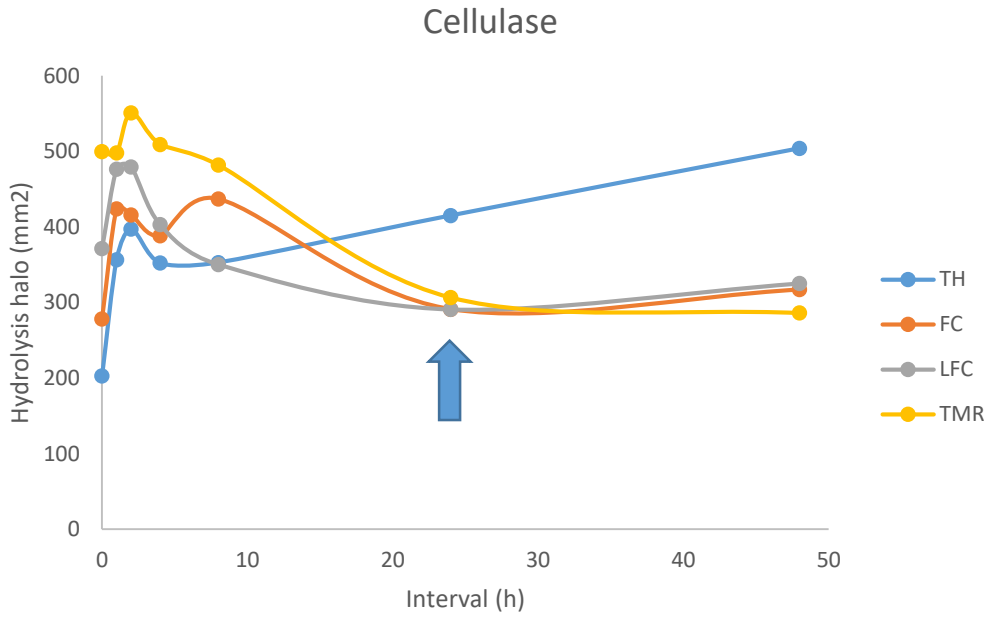




	INTERVAL																P		
AMYLASE	0			1			2			4			8			24			48			SEM	Diet	Interval	D*I
TH	135,61	a	A	197,81	b	B	220,65	c	B	231,88	cd	A	260,13	e	B	200,03	b	C	234,38	d	D	3,236			
FC	135,84	a	A	180,91	c	A	183,75	c	A	216,99	d	A	233,37	e	A	141,54	a	A	153,15	b	A	3,203			
LFC	225,93	c	B	265,25	d	D	280,86	ef	C	284,74	f	C	272,12	de	B	171,65	a	B	186,63	b	B	3,527	<0,001	<0,001	<0,001
TMR	223,40	c	B	244,21	d	C	269,51	e	C	268,55	e	B	242,63	d	A	171,20	a	B	202,42	b	C	3,542			
SEM	4,478			4,393			4,726			4,747			4,193			2,885			3,997						
P	<0,001			<0,001			<0,001			<0,001			<0,001			<0,001			<0,001						

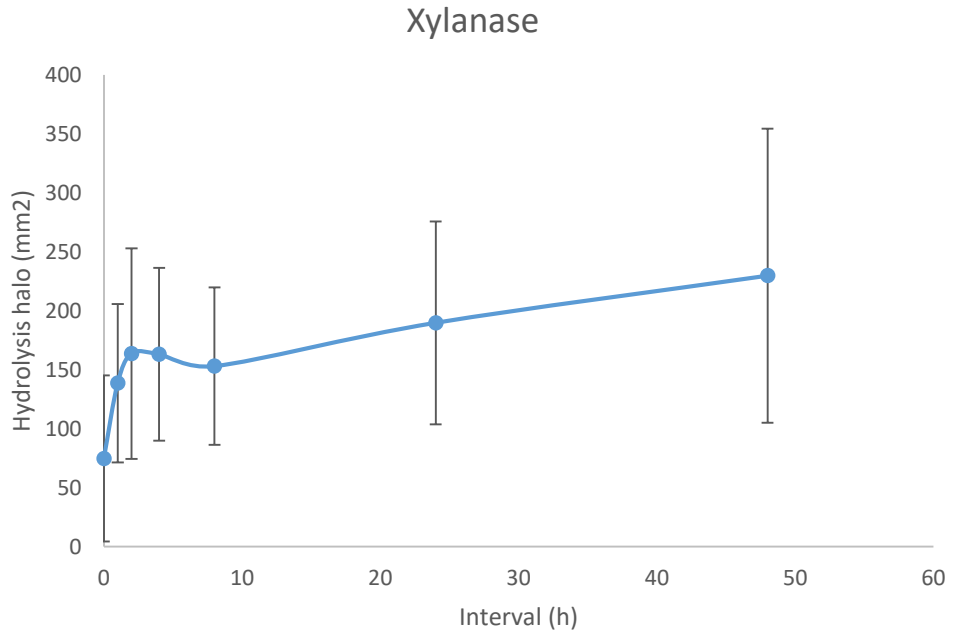
RESULTS AND DISCUSSION - 5

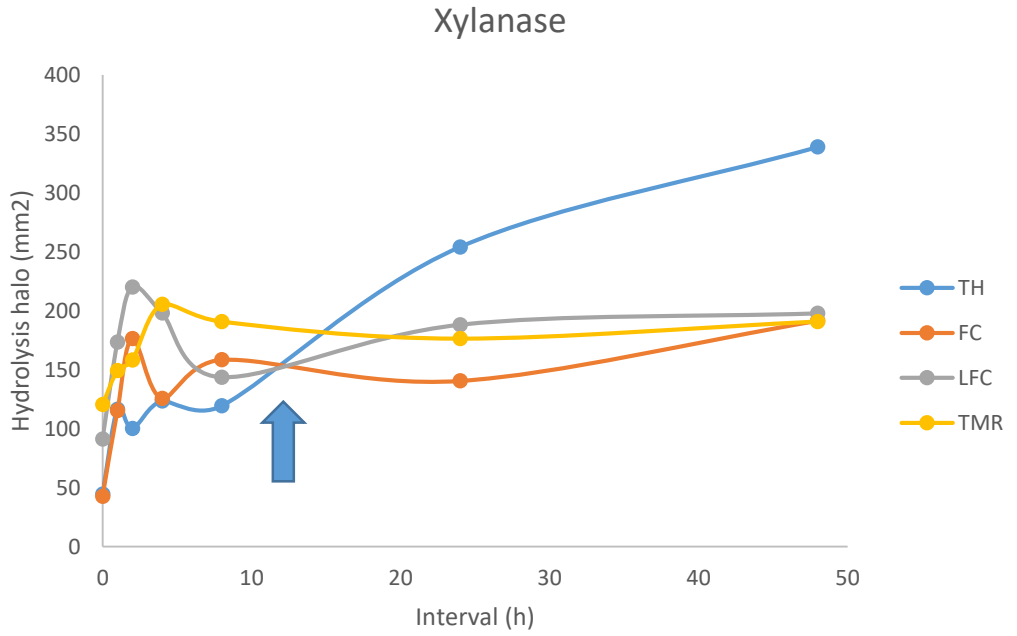




	INTERVAL																		P		
CELLULASE	0			1			2			4			8			24			48			SEM	Diet	Interval	D*I
TH	202,79	a	A	356,85	b	A	397,45	c	A	352,34	b	A	353,05	b	A	415,26	c	B	504,15	d	C	7,767	<0,001	<0,001	<0,001
FC	278,33	a	B	423,87	de	B	415,96	d	B	388,59	c	B	437,18	e	B	291,19	a	A	317,13	b	B	4,639			
LFC	371,42	d	C	476,51	f	C	479,53	f	C	403,13	e	B	350,46	c	A	290,91	a	A	325,31	b	B	5,031			
TMR	499,92	cd	D	497,94	cd	D	550,85	e	D	509,27	d	C	481,88	c	C	306,67	b	A	286,23	a	A	6,246			
SEM	9,917			5,301			5,866			5,760			6,665			8,519			9,716						
P	<0,001			<0,001			<0,001			<0,001			<0,001			<0,001			<0,001						

RESULTS AND DISCUSSION - 6





	INTERVAL																		P		
XYLANASE	0			1			2			4			8			24			48			SEM	Diet	Interval	D*I
TH	44,71	a	A	116,41	b	AB	100,24	b	A	123,48	b	A	119,42	b	A	254,15	c	C	338,86	d	B	11,974			
FC	42,60	a	A	115,44	b	AB	176,52	cd	B	125,78	b	A	158,43	c	B	140,56	bc	A	191,56	d	A	7,016	<0,001	<0,001	<0,001
LFC	91,29	a	B	173,37	bc	C	220,19	d	C	198,02	cd	B	143,63	b	AB	188,14	cd	B	197,77	cd	A	5,698			
TMR	120,54	a	C	149,44	b	BC	158,29	b	B	205,39	cd	B	190,73	c	C	176,37	bc	AB	190,85	bc	A	7,493			
SEM	8,302			7,914			10,518			8,640			7,864			10,140			14,686						
P	<0,001			0,002			<0,001			<0,001			<0,001			<0,001			<0,001						

CONCLUSIONS

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- RFs of different dietary origin significantly **modify their enzymatic activities during the *in vitro* incubation**

- Modifications depend from **characteristics of the diet** originating the RFs and on the **fermentation interval** considered

- Rumen fluids from **high fiber diets improved** their enzymatic activities at both 24 and 48 h of fermentation
Low fiber diet derived rumen fluids showed final levels of enzymatic activities **lower** than the initial ones

Xyl was improved by the priming process in all cases

- The inocula enzymatic activities differences were minimized at:
8 h for both amylase and xylanase
24 h for cellulase

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Thank you

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