Effect of fresh hemp and savory leaves on feed intake and rumen fermentation: *in vitro* and *in vivo* trials

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Introduction

The pharmaceutical and herbal sectors produce large amounts of by-products, potentially are usable in dairy cows' nourishment

Few studies have been conducted on by-products

But are we sure that certain plants or herbs have an effect on the rumen fermentation or on methane emissions as whole products?

Investigated substrates

Cannabis sativa L. → Hemp leaves (HL)

Variety: **Futura 75**
Cultivation: Research center, Italy
Harvesting time: **July-August 2022**
Harvest part: **top leaves & pre-blooming flower**
Stocking temperature: **- 20°C**

Satureja hortensis L. → Savory leaves (SL)

Cultivation: Paodva, Italy
Harvesting time: August-September 2022
Harvest part: top leaves
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Aims

Experiment 1.
***In vitro* study**

Evaluation of **HL** and **SL** *in vitro* on ruminal parameters, using *Lolium multiflorum* L. as control test (CTRL)

Experiment 2.
In vivo

Evaluation *in vivo* of **HL** and **SL** **in a TMR** on the rumen fluid modification with a Latin Square design 3×3

Experiment 3.
In vitro* after *in vivo trial

Evaluation *in vitro* of ruminal parameters, using the **rumen fluid** collected *in vivo* (Experiment 2)

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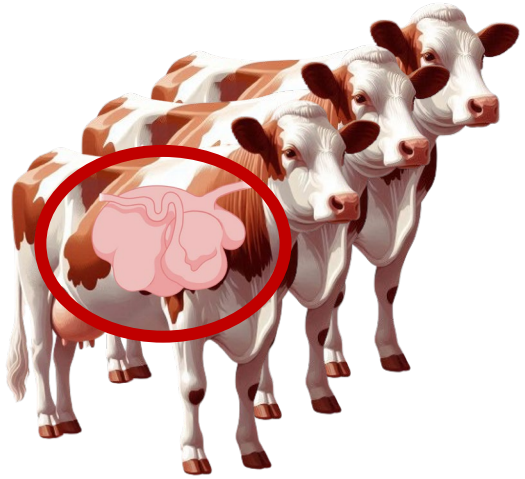
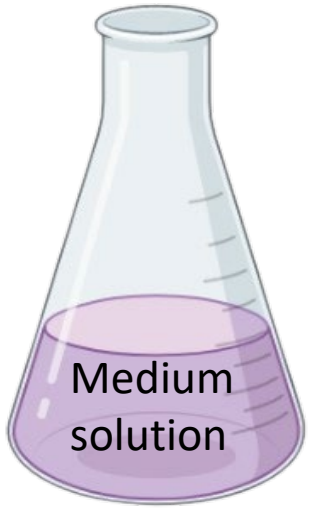
Materials and Methods

Experiment 1 – Feed test *in vitro*

3x
Rumen Fluids



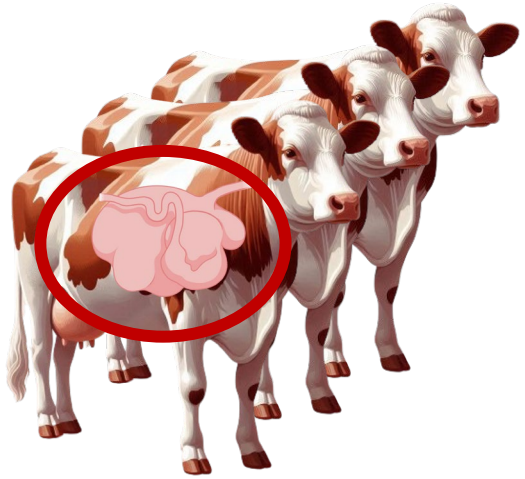


50 mL

100 mL

Menke and Steingass, 1988

Experiment 1 – Feed test *in vitro*



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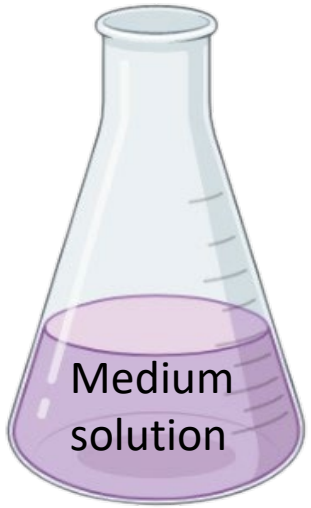

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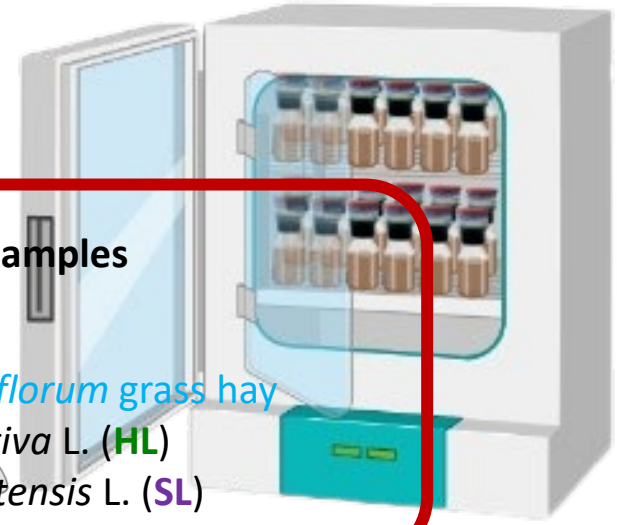
100 mL

Gas sampling
at 48 h



Samples

1 gram of
- ✓ *Lolium multiflorum* grass hay
- ✓ *Cannabis sativa* L. (**HL**)
- ✓ *Satureja hortensis* L. (**SL**)

3 replicates per each feed

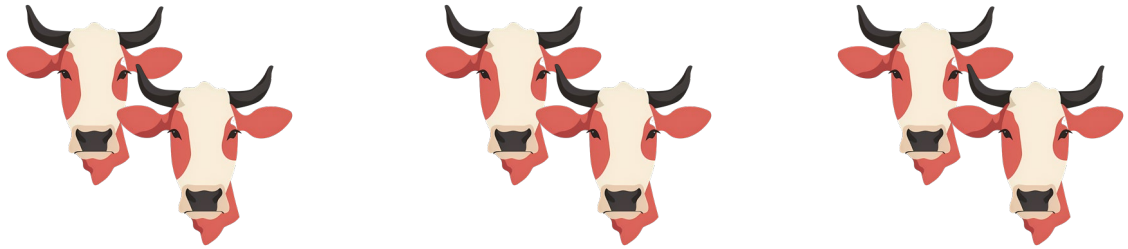
Completely electrified system

48 hours

Menke and Steingass, 1988

Experiment 2 - Feed test *in vivo*

- ✓ 3 groups of 2 animals
- ✓ 3 periods of 14 days
- ✓ 3 rumen fluid collections



1	CTRL	TMR+HL	TMR+SL
2	TMR	TMR	TMR
3	TMR	TMR	TMR

Each animal was fed with all the 3 treatments in the 3 periods.

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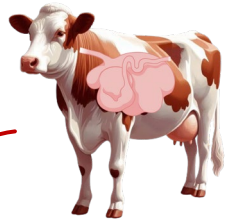


HL + 0.5 kg/d **HL + 1.0 kg/d** **HL + 1.5 kg/d**
SL + 0.2 kg/d **SL + 0.4 kg/d** **SL + 1.0 kg/d**

1 2 3 4 5 6 7 8 9 10 11 12 13 14

Transition period **Treatment administration** **Transition period**

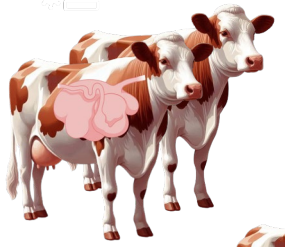
For each period:

1 group animal fed with TMR (CTRL)
1 group animal fed with TMR + **HL**
1 group animal fed with TMR + **SL**

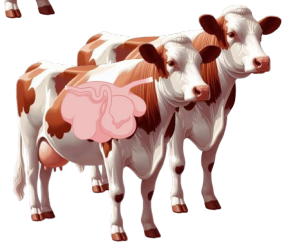
Increasing doses

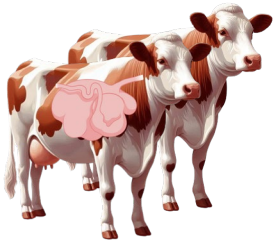
Experiment 3 - Rumen fluid activity *in vitro*

2 CTRL
Rumen fluid



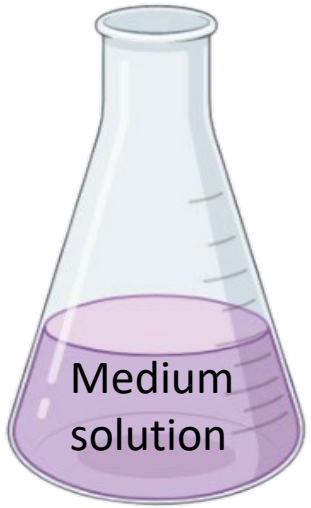
2 TMR+HL
Rumen fluid





2 TMR+SL
Rumen fluid

The rumen fluids, adapted or non-adapted, were collected from the animals raised for the *in vivo* experiment (Experiment 2).



50 mL

100 mL

3 replicates per each rumen fluid

Menke and Steingass, 1988

Experiment 3 - Rumen fluid activity *in vitro*

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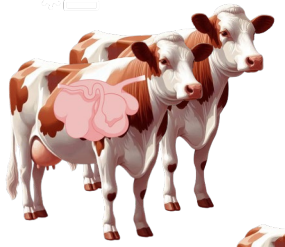
Rumen fluid



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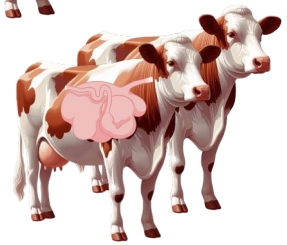

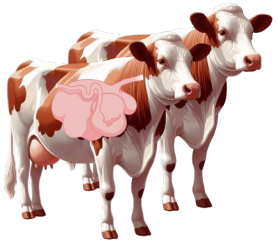

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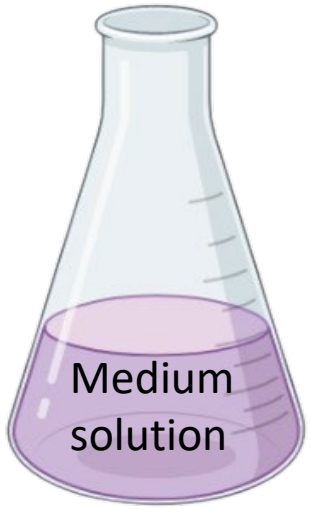

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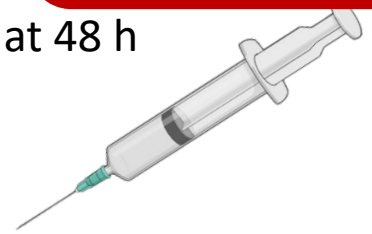

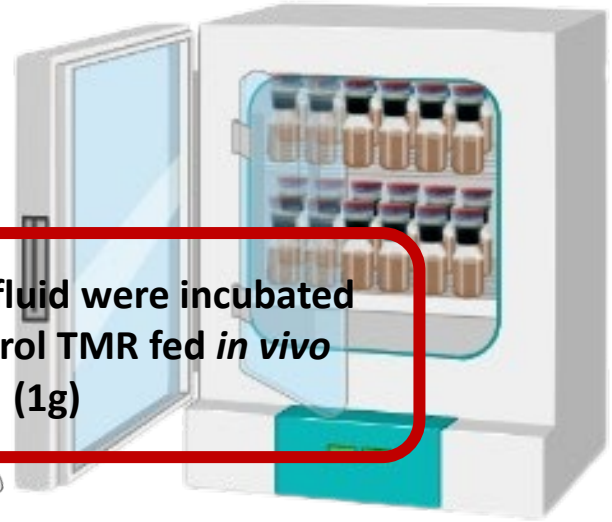

All the rumen fluid were incubated with the control TMR fed *in vivo* (1g)

Gas sampling
at 48 h

48 hours

Completely
electrified
system

3 replicates per each rumen fluid

Menke and Steingass, 1988

Statistical model – Experiment 1

$$y_{ijklm} = \mu + Treatment_i + Cow_j + Position_k(Incubator_l) + e_{ijklm}$$

- Fixed effects
  - $Treatment_i$= 3 treatments

    (*Lolium multiflorum*, *Cannabis sativa*, *Satureja hortensis*)
- Random effects
  - Cow_j= 3 dairy cows
  - $Position_k(Incubator_l)$ = 2 position per 2 incubators
    - position effect nested in the incubator

Treatments orthogonal contrasts

Lolium multiflorum, control test

VS

- Cannabis sativa (HL)
- Satureja hortensis (SL)

Statistical model – Experiment 2 and 3

$$y_{ijklmn} = \mu + Animal\ group_i + Period_j + Diet\ in\ vivo_k + Cow_l(Animal\ group_m) + e_{ijklmn}$$

- Fixed effects
  - $Animal\ group_i$= 3 groups of animals
  - $Period_j$= 3 periods
  - $Diet\ in\ vivo_k$= 3 diets administrated to the groups
- Random effects
  - $Cow_l(Animal\ group_m)$ = 2 cows per 3 groups
    - cow effect nested in the animal group

Treatments orthogonal contrasts

Non-adapted rumen fluid

Animal fed with TMR (total mixed ratio, **CTRL**)

VS

Adapted rumen fluid

Animal fed with **TMR+HL**

Adapted rumen fluid

Animal fed with **TMR+SL**

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Ph.D. COURSE ANIMAL & FOOD SCIENCE UNIVERSITY OF PADOVA

Results

Feed composition

Chemical composition of feeds (% of DM)

Feeds	DM, %	CP	EE	NDF	ADF	ADL	Ash	AIA
Lolium multiflorum L.[1]	89.9	8.08	1.67	59.0	33.1	4.41	7.07	0.52
Cannabis sativa L. (HL)	92.0	19.6	7.12	30.5	15.8	5.29	13.9	0.09
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DM: Dry Matter.

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EE: Ether Extract.

NDF: Neutral Detergent Fiber.

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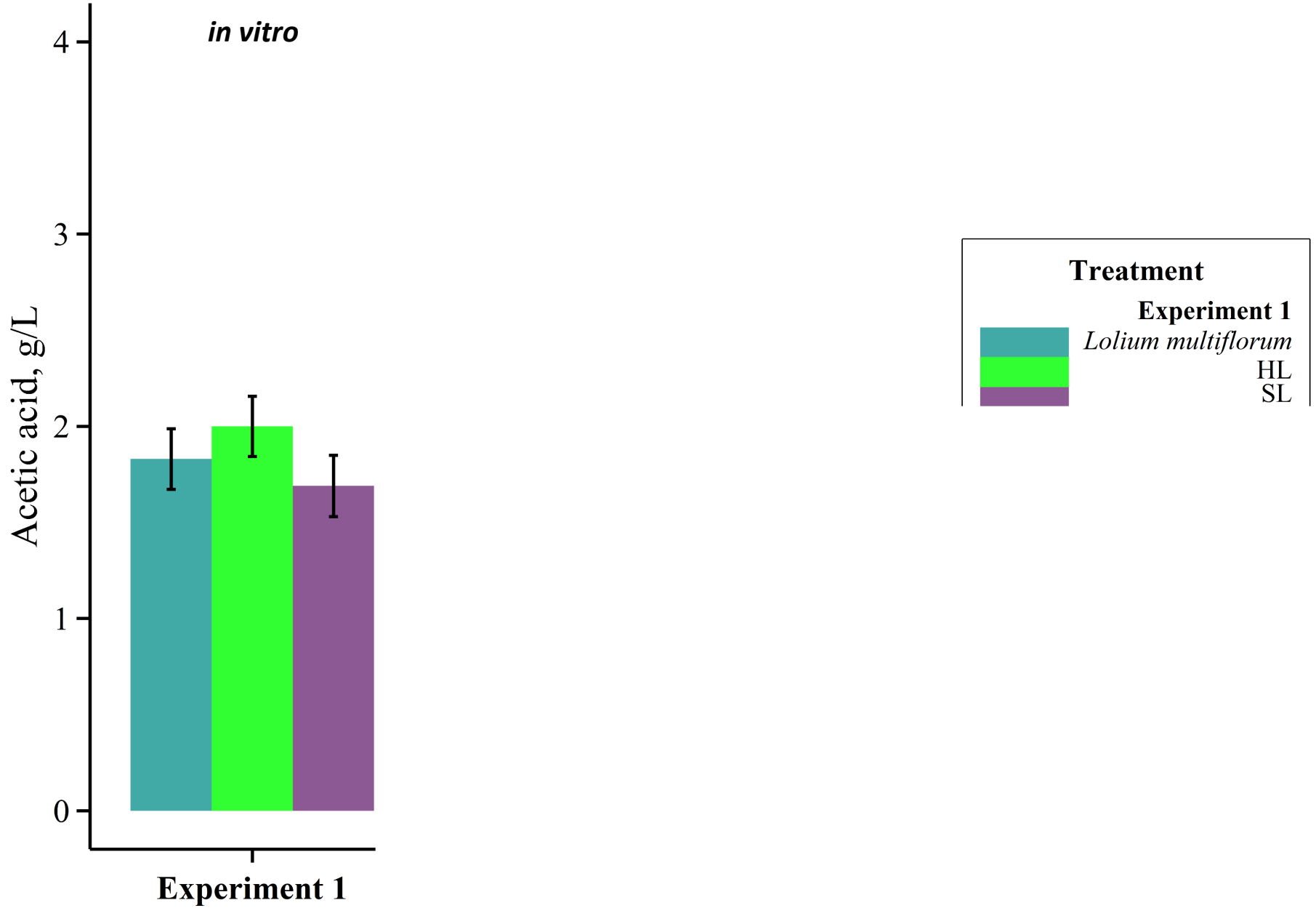
Ruminal parameters

Volatile fatty acids (VFA, g/L)



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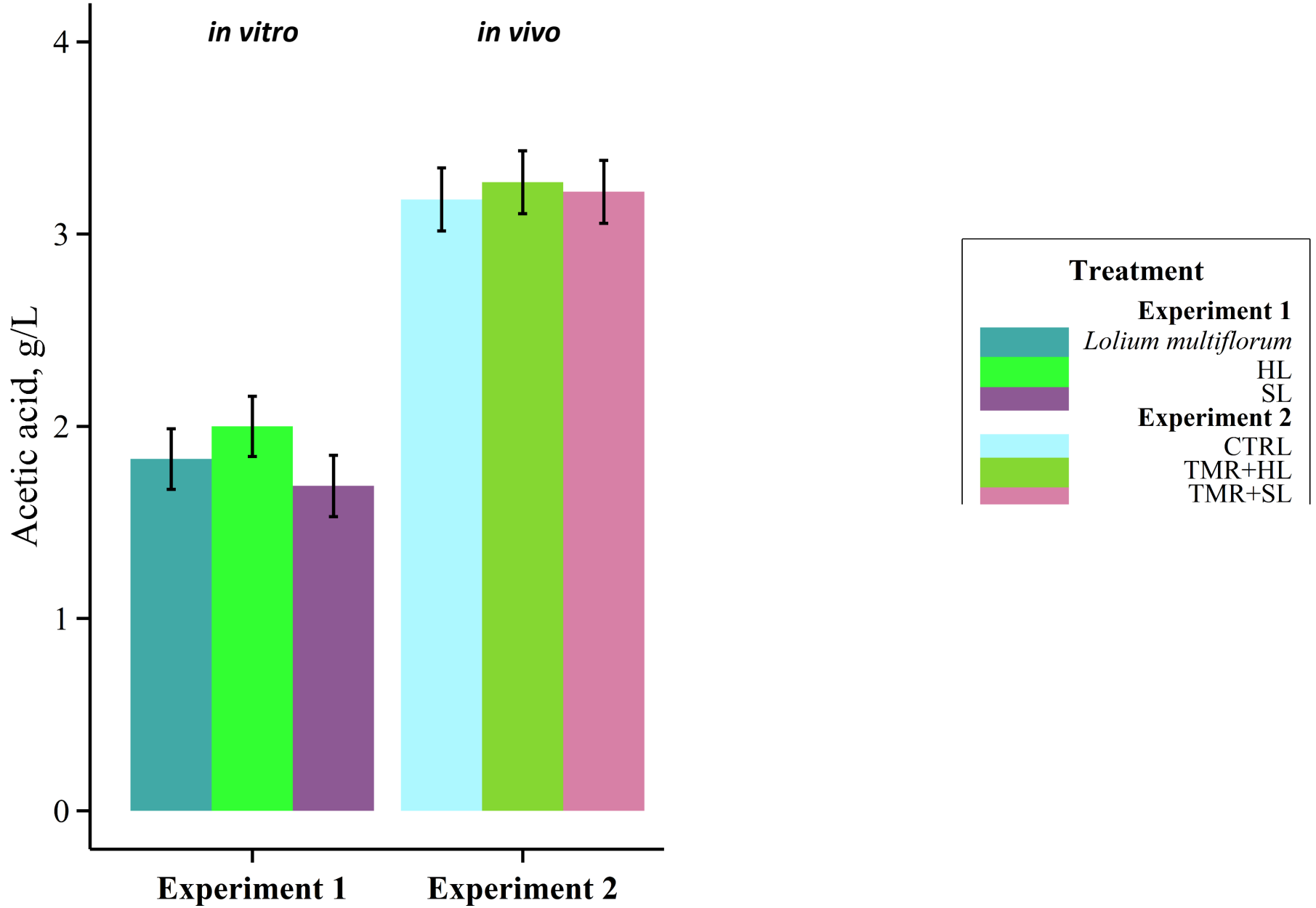


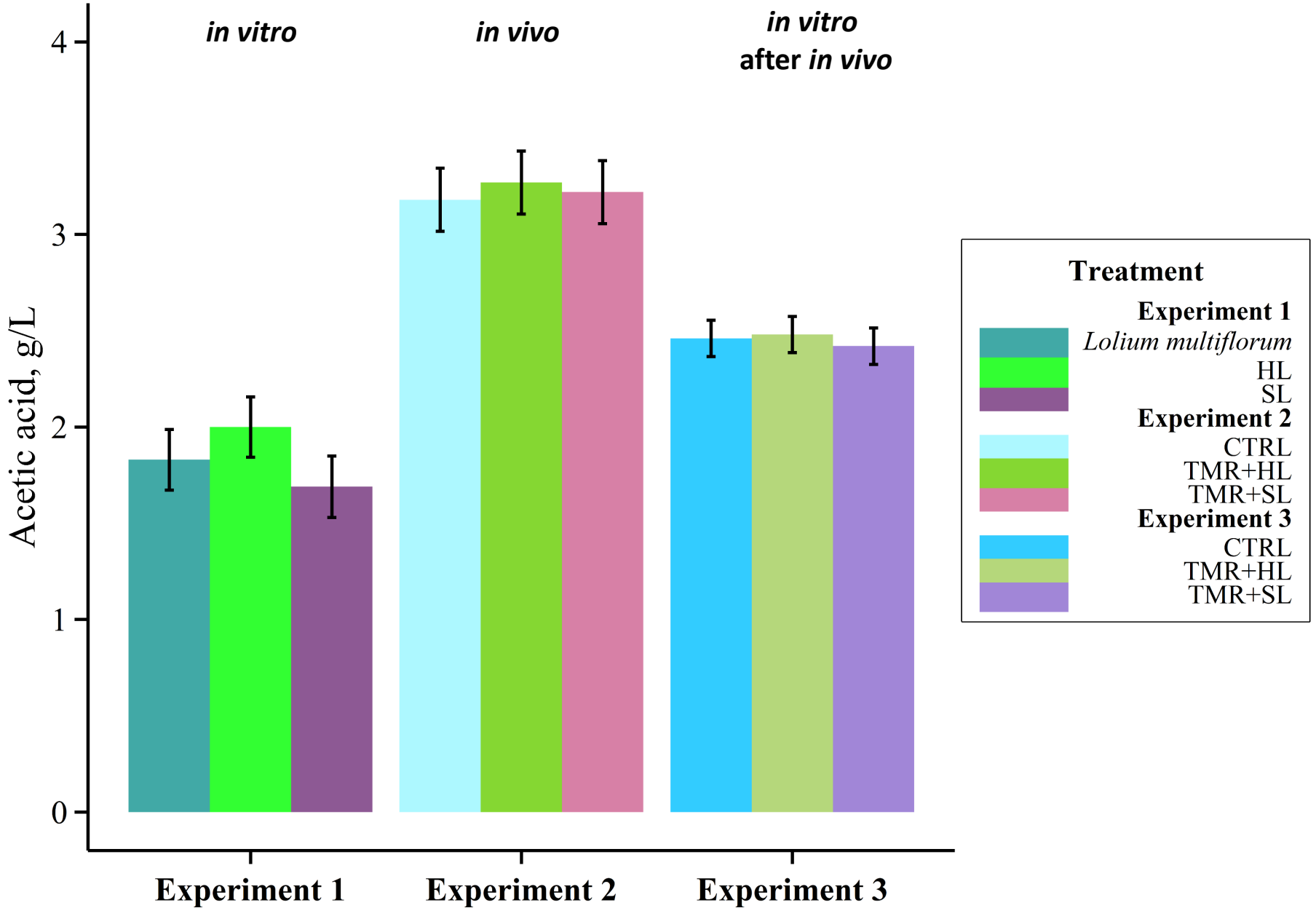
Volatile fatty acids (VFA, g/L)

Experiment 1
Experiment 3

The values referes to the end of the *in vitro* fermentation (48 h)

Experiment 2

The value referes to rumen fluid collected to *in vivo* trials



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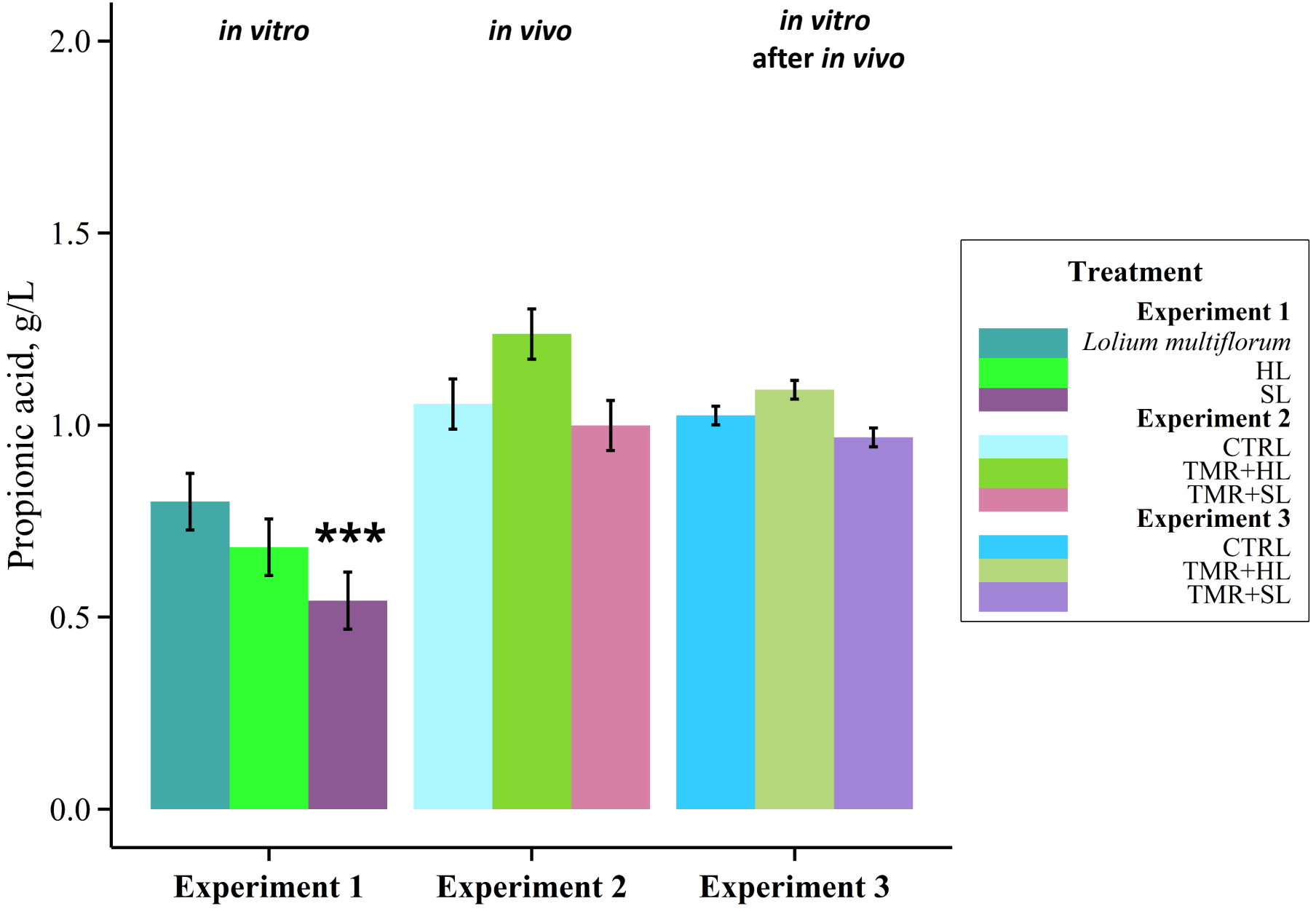

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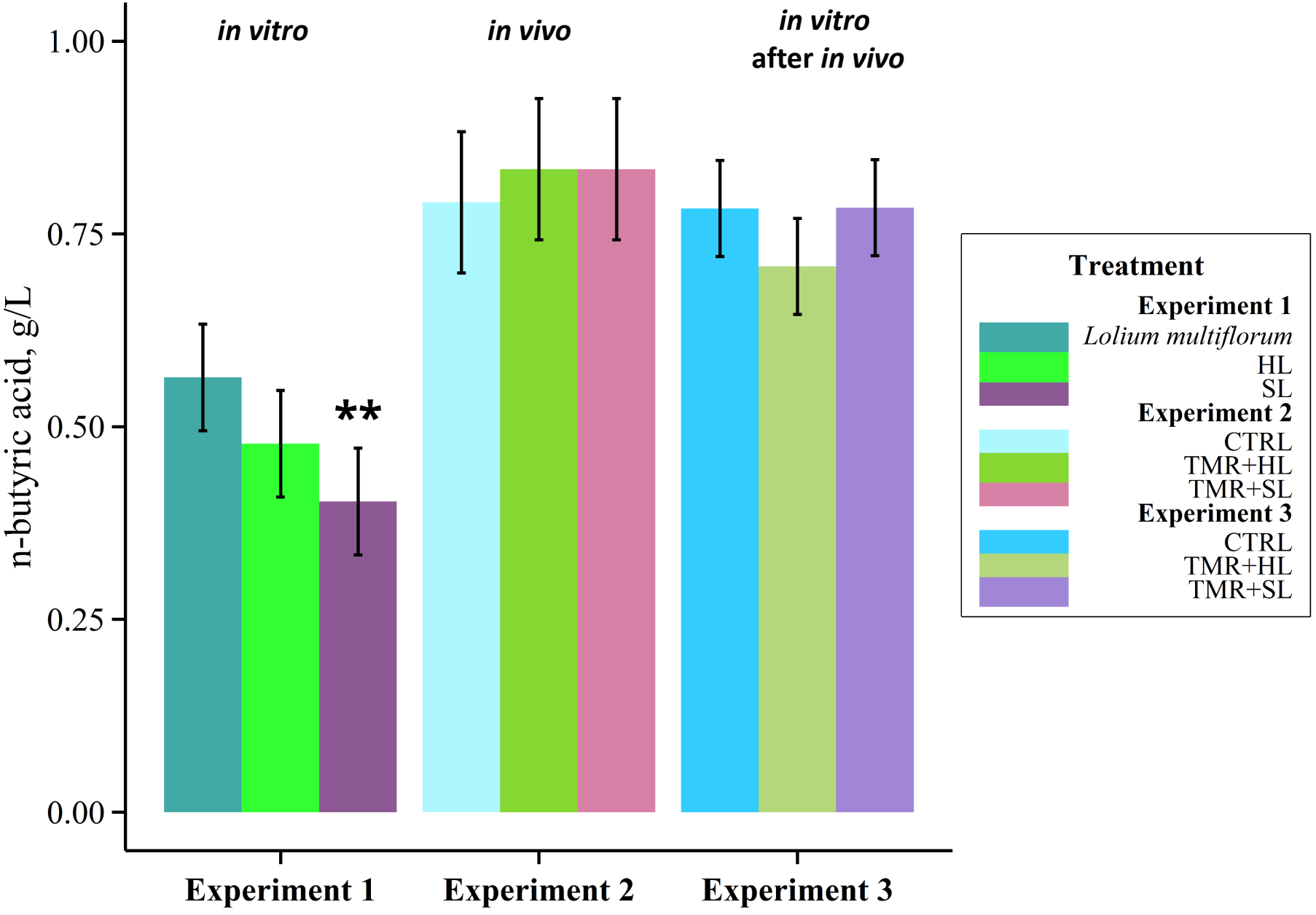
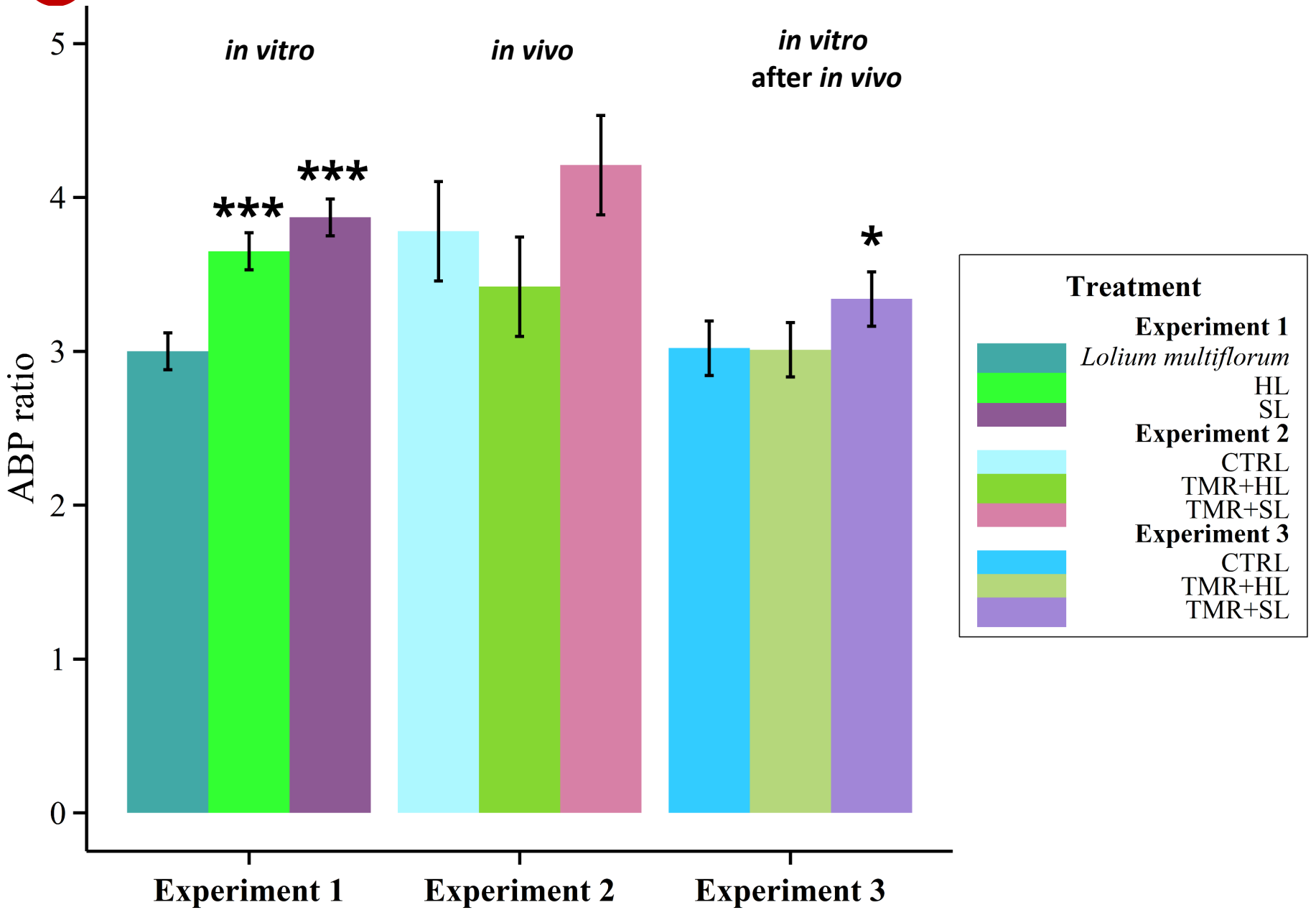
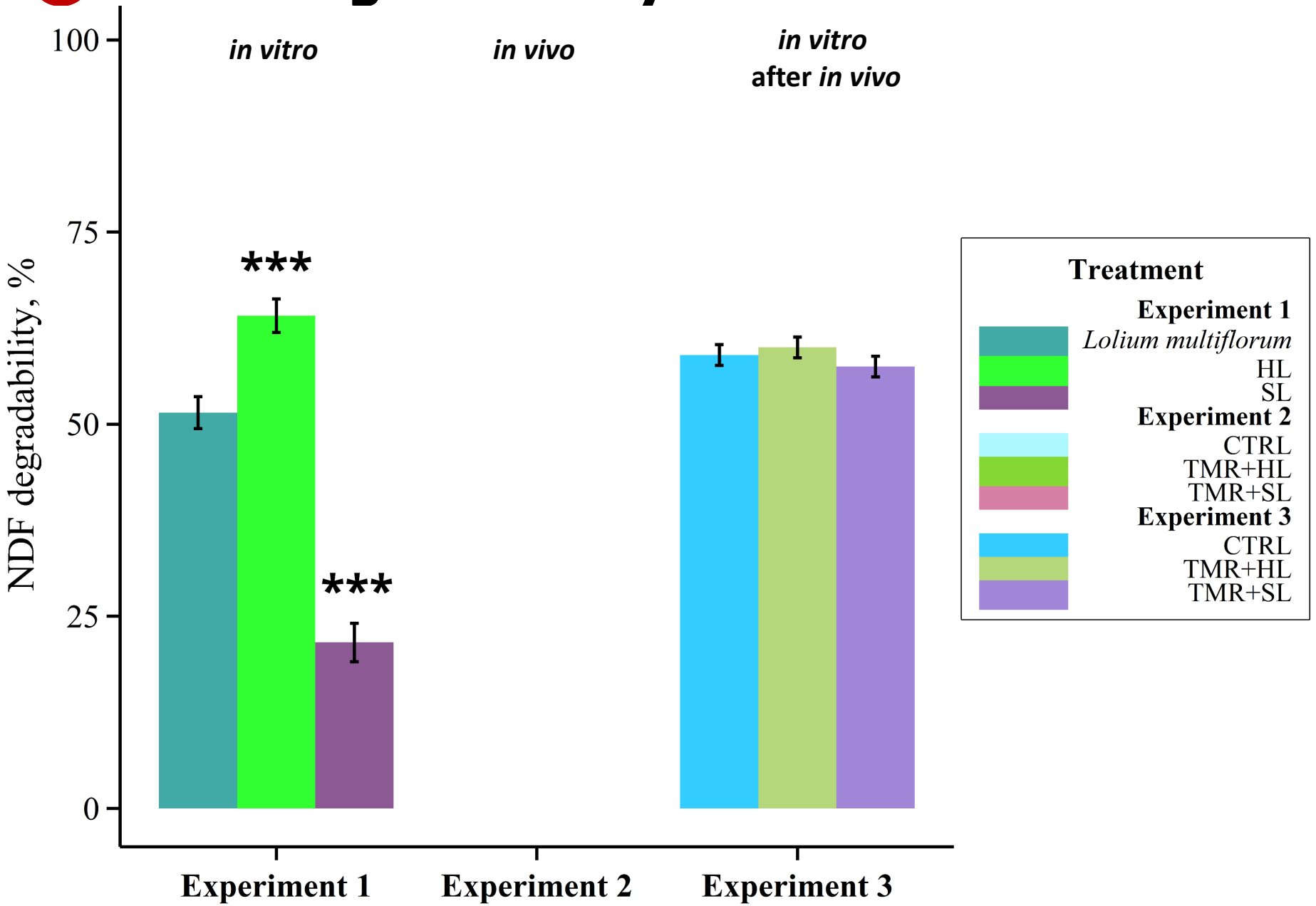


ABP ratio

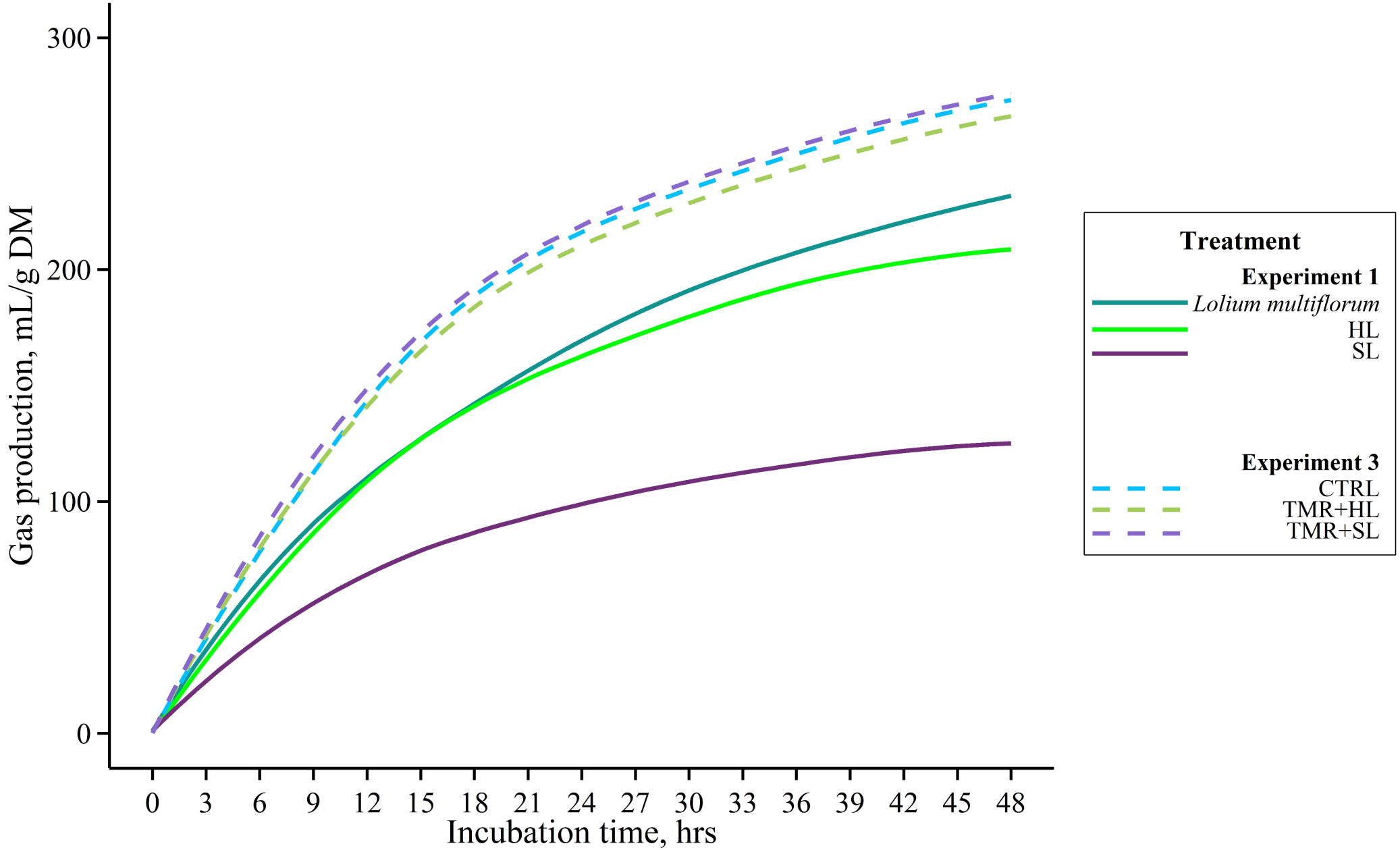

in vitro
in vivo
in vitro
after in vivo
ABP ratio
0
1
2
3
4
5
***
***
*
Experiment 1
Experiment 2
Experiment 3
Treatment
Experiment 1
Lolium multiflorum
HL
SL
Experiment 2
CTRL
TMR+HL
TMR+SL
Experiment 3
CTRL
TMR+HL
TMR+SL

Post-fermentation traits

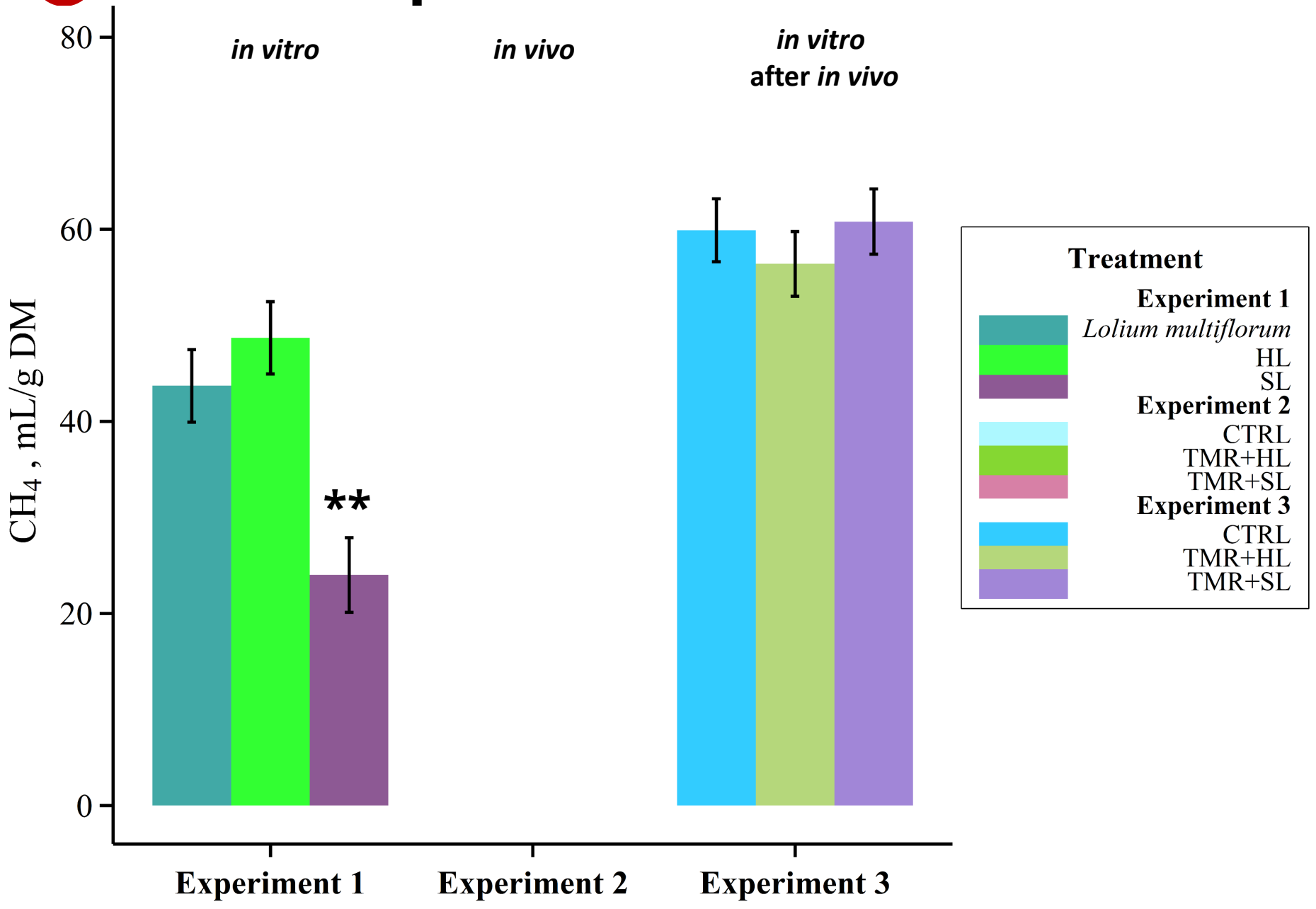
NDF degradability at 48 h



Gas production in 48 h



Methane production at 48 h



Conclusions

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- ✓ **Savory Leaves** changed the amount of **propionic and n-butyric acids**, and reduced both **TGP** and **methane** production (mL/g DM).

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- ✓The pure substrates (**Hemp** and **Savory** Leaves) influenced significantly the **ABP ratio**, the **NDF degradability**.
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- ✓All the traits evaluated for Exp. 2 showed no significant differences.

Conclusions

- ✓The pure substrates (**Hemp** and **Savory** Leaves) influenced significantly the **ABP ratio**, the **NDF degradability**.
- ✓**Savory Leaves** changed the amount of **propionic and n-butyric acids**, and reduced both **TGP** and **methane** production (mL/g DM).
- ✓All the traits evaluated for Exp. 2 showed no significant differences.
- ✓The adapted or non-adapted rumen fluid (Exp. 3), showed no significant differences in the traits.

Future challenges

→ More *in vivo* study to evaluate the best amount of herb to show an effect on the rumen fluid

→ Necessity to study and characterize the herbs and the potential inhibitors of methane production

→ Testing by-products for the implementation in animal feeding strategies

Thank you for the attention

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Acknowledgments
This work is a part of *BIRD213117/21* (founded by the University of Padua).

Treatments composition

Chemical composition of treatments (% of DM)

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Satureja hortensis L. (SL)	89.6	8.43	5.41	60.5	45.6	17.8	7.2	0.29
Total mixed ratio (TMR)[2]	90.6	14.7	3.41	41.4	21.1	4.11	7.52	0.57

DM: Dry Matter.

CP: Crude Protein.

EE: Ether Extract.

NDF: Neutral Detergent Fiber.

ADF: Acid Detergent Fiber.

ADL: Acid Detergent Lignin.

AIA: Acid Insoluble Ash

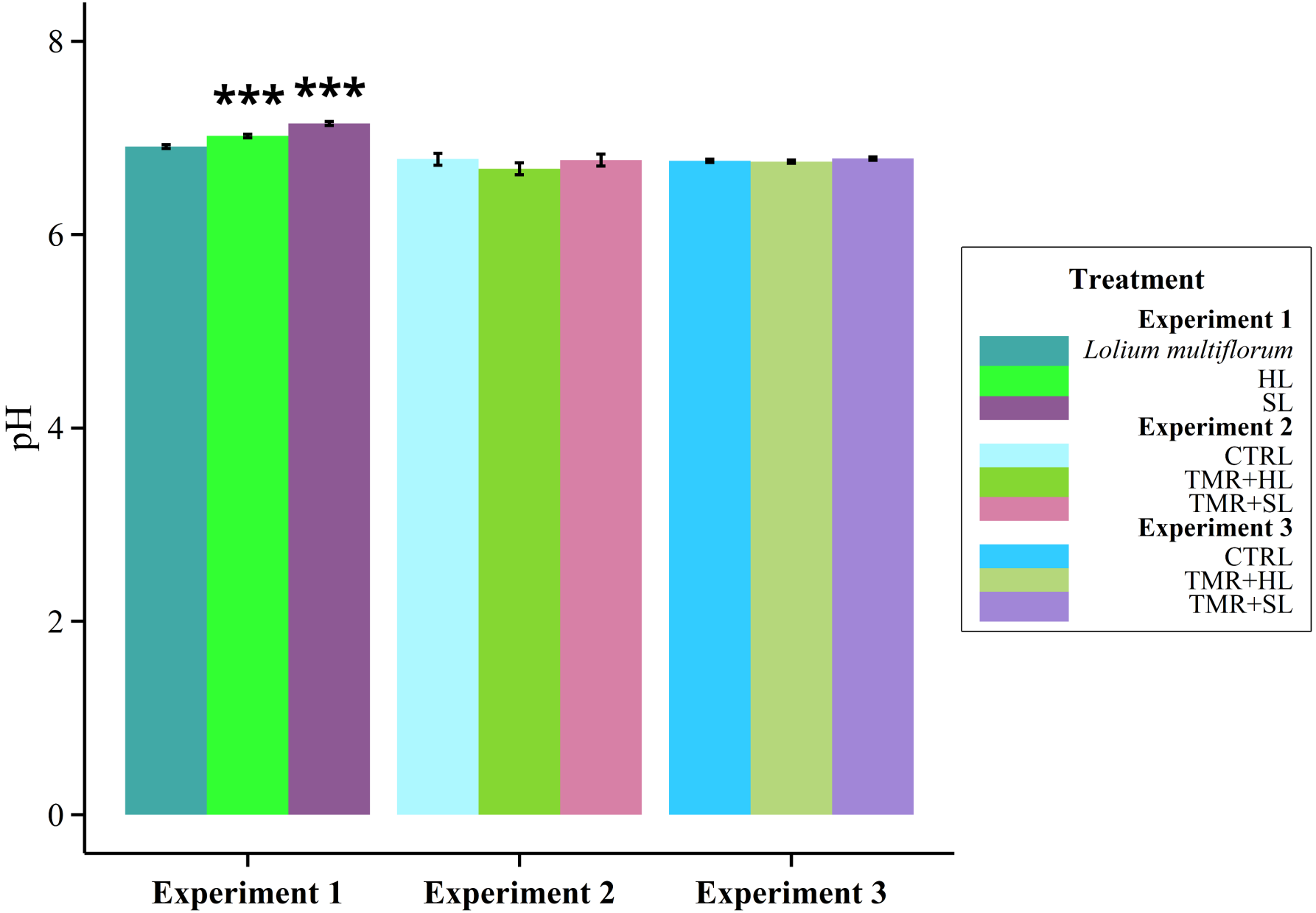
[1]*Lolium multiflorum* L. was used in the *in vitro* test as a control for Experiment 1

[2]Total mixed ratio (TMR) was used in the *in vivo* and *in vitro* test as a control for Experiment 2 and 3.

Total mixed ratio composition

Ingredients	Quantity, % on DM
Energy mix	6.89
Protein mix	2.99
Sorghum silage	2.32
Alfalfa hay	2.27
Grass silage	1.92
Corn gluten	1.54
Molasses	0.97
Mixed hay	0.91
Soybean meal	0.45
Linseed + corn germ	0.27
TOTAL	**20.53**

pH


pH
8
6
4
2
0
*** ***
Experiment 1
Experiment 2
Experiment 3
Treatment
Experiment 1
Lolium multiflorum
HL
SL
Experiment 2
CTRL
TMR+HL
TMR+SL
Experiment 3
CTRL
TMR+HL
TMR+SL