Effects of cashew nutshell liquid on milk production and methane emission of dairy cows

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Background

- Enteric fermentation in ruminants is thought to be one of the largest sources of global anthropogenic methane emissions (US EPA, 2005; FAO, 2007).

- Mitigating methane production from ruminant animals has long been targeted.

- Methane mitigation studies involving ruminants have proposed several approaches based on various aspects of animal feed and management (Tamori et al., 2021).

Enteric methane mitigation options

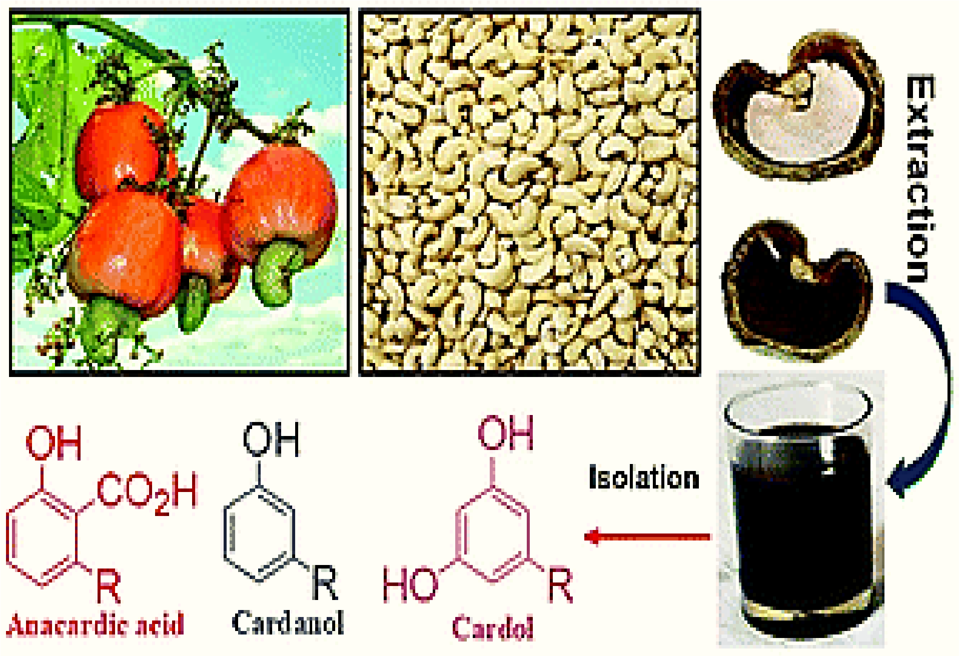
- *Increased animal productivity*
- *Selection of low-methane-producing animals*
- *Diet formulation*
  - Levels of feed and concentrate intake, sources, and processing
  - Lipid supplementation, etc.
- *Forages*
  - Increase digestibility
  - Pastures and grazing management, etc.
- *Action on rumen fermentation*
  - Ionophores
  - Direct-fed microbials, etc.
- *Early-stage mitigation strategies*
  - Immunization against methanogens
  - Rumen defaunation, etc.

Source:Beauchemin et al., 2022

Cashew nutshell liquid as a natural anti-microbial inhibitor

- Cashew (*Anacardium occidentale)*
  - * a perennial tree crop
  - * widely grown in tropical countries

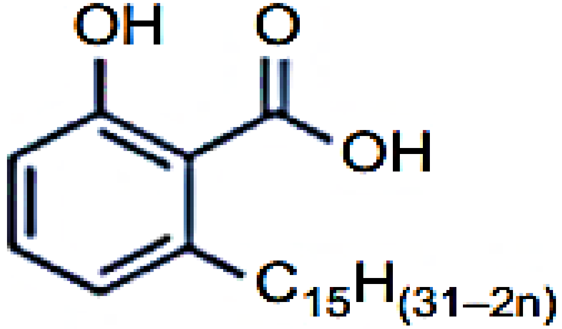
- Cashew nut shell liquid (CNSL)
  - * a dark brown viscous liquid
  - * has numerous industrial applications such as friction linings, paints, varnishes, etc.
  - * contains anacardic acid, cardol, and its methyl derivatives.



Mgaya, J., Shombe, G. B., Masikane, S. C., Mlowe, S., Mubofu, E. B., & Revaprasadu, N. (2019). Cashew nut shell: a potential bio-resource for the production of bio-sourced chemicals, materials and fuels. *Green Chemistry*, *21*(6), 1186-1201.

Anacardic acid in CNSL has antimicrobial properties that can potentially mitigate enteric methane production

Action	Reference
* Propionate enhancer	Van Nevel et al., 1971
* Inhibit gram-positive bacteria, including bacilli and staphylococci	Kubo et al., 1993
* Specific target bacterial species: *B. fibrisolvens, Treptonema bryantii, E. ruminantium, Staphylococcus aureus, R. albus, and R. flavefaciens*	Watanabe et al., 2010; Shinkai et al., 2012



Anacardic acid

Studies on methane mitigating potential of CNSL

Study	Inclusion range	Reduction (%)	Reference
In vitro	* Raw and heated CNSL at 500 µg/mL rumen fluid * 0, 50, 100 or 200 µg/mL rumen fluid	* Raw CNSL 57% reduction * Heated CNSL 9% reduction * 70 % reduction at 200 µg/mL * 50 % reduction at 100 µg/mL * 36 % reduction at 50 µg/mL	Watanabe et al., 2010
In vivo	* On dry cows * 30 g/day or 0.3% of DMI equal to 200 µg/mL rumen fluid	* CH_4/DMI: 19%	Shinkai et al., 2012

* *In vitro*, showed a linear decrease in methane production
* *In vivo*, even a low-level CNSL is expected to exhibit an inhibitory effect

Research gap

- The effect of CNSL on methane emission and milk production of dairy cows in a barn environment is unknown.
- The use of a sniffer approach to assess the impact of methane inhibitors on dairy cows is limited.

Points to clarify

- Whether a low level of CNSL supplementation will affect methane production in dairy cows under free stall barn conditions?
- Whether or not the sniffer method is applicable to detect methane mitigating effects by CNSL?

Objectives

Evaluate the efficacy of CNSL at a low-level supplementation to lactating dairy cows in a free stall barn in terms of:

- Enteric methane emission
- Milk production and composition
- Rumen fermentation
    - *In vivo*
    - *In vitro*

Methodology

Animals

- ❑ Ten lactating Holstein cows
- ❑ Parity: 2.0 ± 0.67
- ❑ Rearing condition:
  - * Free-stall barn
  - * Individual feed box that can control entrance and record eating time and daily intake
  - * AMS with the free-cow traffic system

Experimental design and Treatments

- ❑ Randomized complete block design with repeated measurements for four weeks.
- ❑ Control (without supplementation)
- ❑ CNSL (top dressing with CNSL at 10 g per day)
- ❑ 1^{st}-week pre-treatment
- ❑ 2^{nd} week to 4^{th} week – CNSL addition

Feeding

- ❑ Partial mixed ration (PMR) – ad libitum
- ❑ Based on Japanese feeding standards for dairy cattle
- ❑ Three times per day (10:00 h; 12:00 h; 15:00 h)
- ❑ Concentrate diet at the milking robot (4 to 8 kg/d)

Experimental diets (PMR and Concentrate)

Ingredients	Partial mixed ration		Nutrient composition, *% DM*	Partial mixed ration		Concentrate
	PMR 1	PMR 2		PMR 1	PMR 2	
Italian ryegrass silage	31.7	25.8	Dry matter (% fresh matter)	53.55	50.85	89.9
Oats hay	10.2	11.0	Crude ash	12.1	14.6	8.9
Alfalfa hay	13.2	14.3	Crude protein	13.1	13.8	17.3
Formula feed	42.8	46.4	NDF	45.9	46.9	20.5
Calcium carbonate	0.49	0.53	Starch	9.5	9.6	39.2
Vitamin mix	0.68	0.74	NFC	24.3	20.7	50.7
Salt	0.32	0.35	Ether extract	4.9	4.0	2.7
Sodium bicarbonate	0.48	0.53	Estimated TDN	69.4	70.2	85.7
Adsorbent	0.23	0.26				

PMR 1: Day 1 to 18; PMR 2: Day 19 to 28

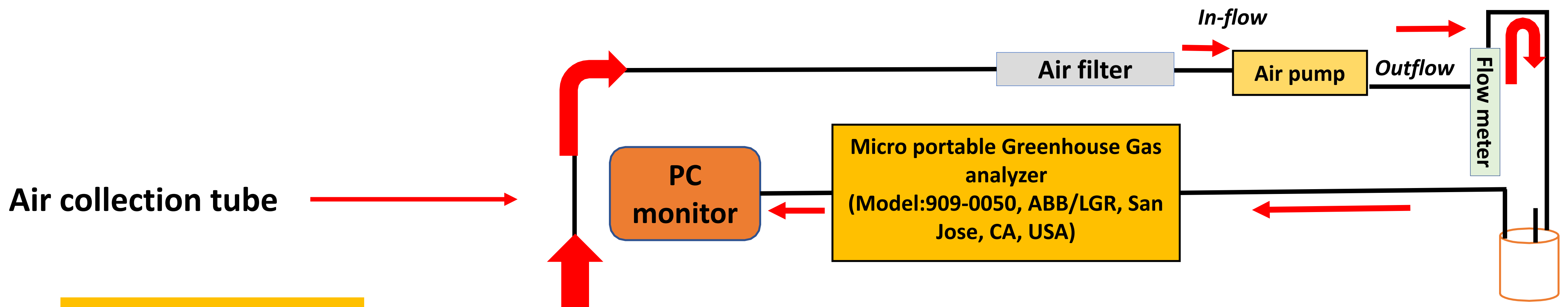
Schedule of CNSL addition, data gathering, and measurement.

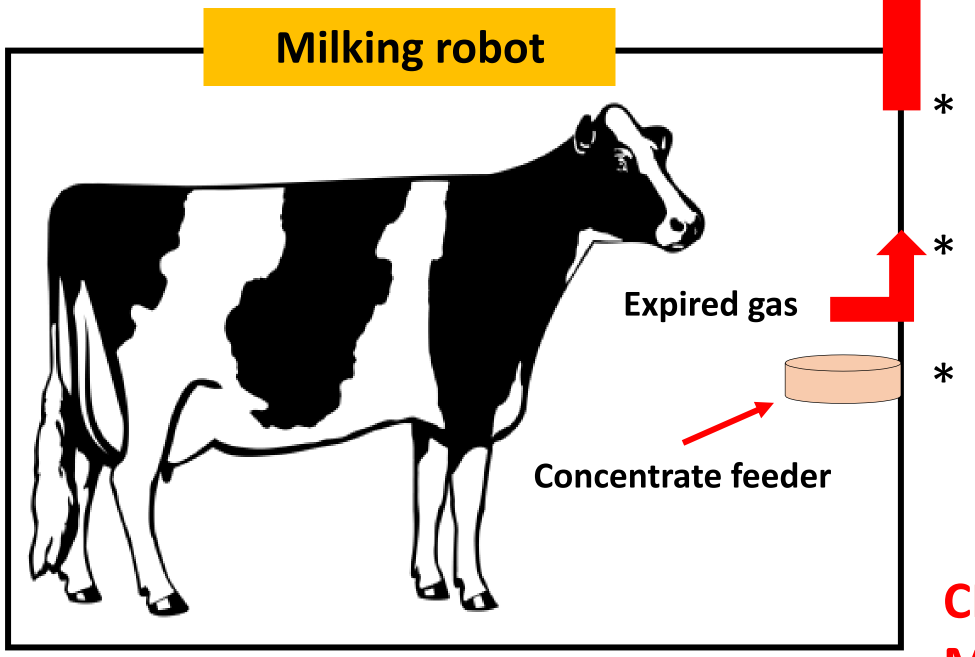
Activities	Week 1	Week 2	Week 3	Week 4
Treatment:				
Top dressing of CNSL				
Measurements:				
DMI				
Milk Yield				
Methane production				
Rumen fermentation				
Milk composition				

* *Composite of milk samples from day 25th to day 27th were used for milk composition analysis*

* *Rumen fluid on day 26 was used for rumen fermentation parameters*

Estimating methane production





Sniffer method for methane production measurement

- Continuous gas sampling from the feed box of the milking robot for 28 days.
- Measuring CO_2 and CH_4 concentration in the gas samples for every second during milking.
- Averaging the daily observation for each week of intake and methane production.

Calculating methane production using the following equation:

CH4 (L/day) = -219 + 20.4 DMI + 3,991 CH4:CO2

MCF (J/100 J) = 3.06 – 0.118 x DMI + 57.3 x CH4:CO2

DMI: dry matter intake (kg/day), CH_4:CO_2: concentration ratio

Based on the regression analysis using a respiration chamber study in NARO Japan (Suzuki et al., 2021)

In vitro batch culture

- ❑ Ten rumen fluid samples from individual cows
  - **Control group n=5*
  - **CNSL group n=5*

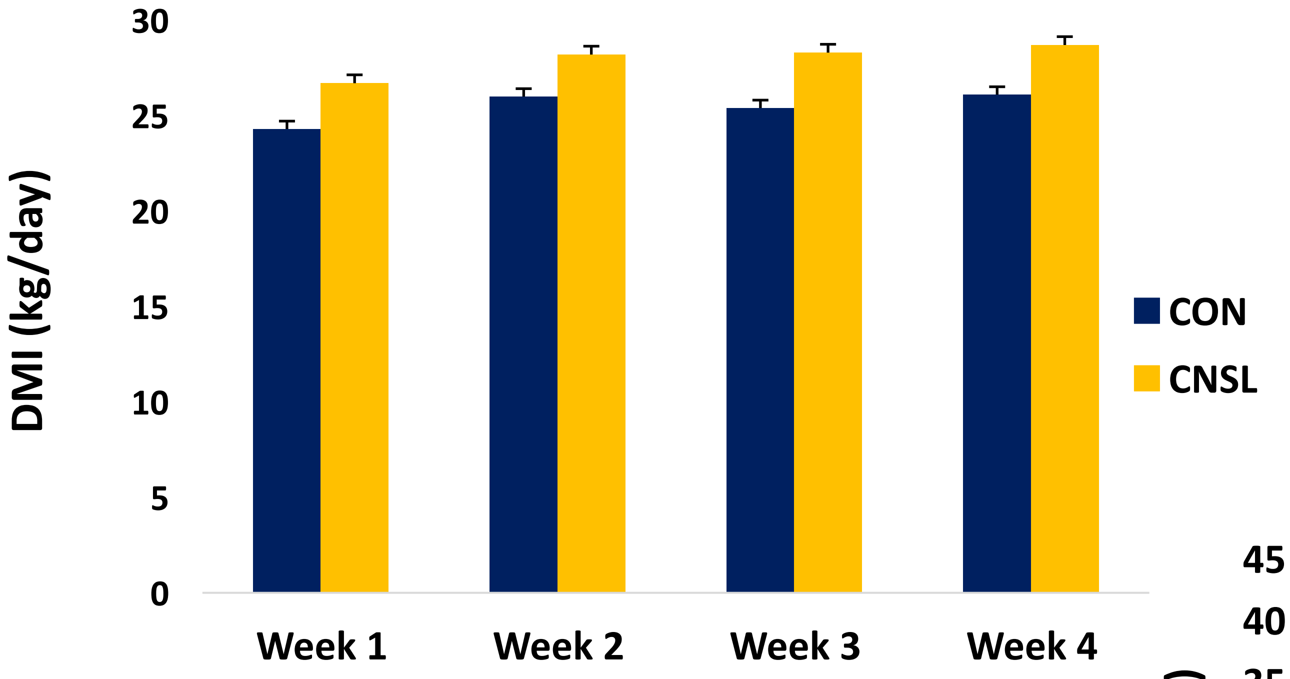
- ❑ 10 mL filtrated rumen fluid mixed with 20 mL pre-warmed McDougall's buffer solution transferred to 50 mL serum bottles

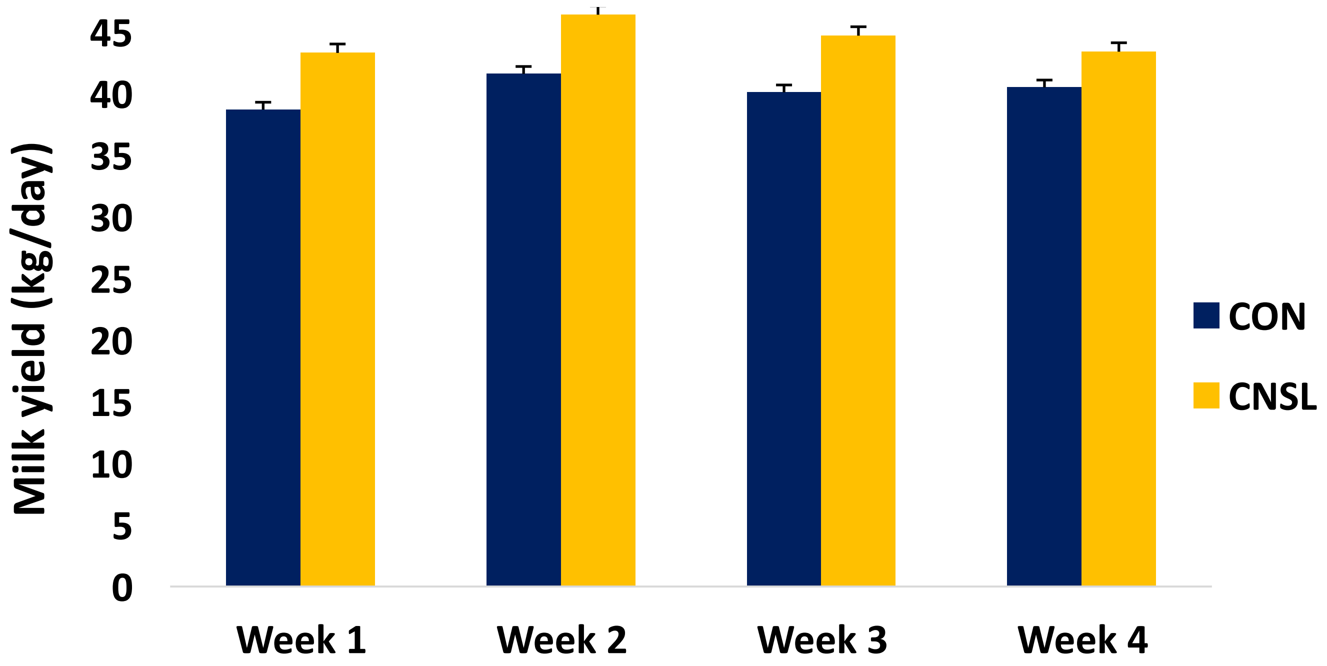
- ❑ The same PMR offered during the feeding trial was used in the incubation.

- ❑ Incubation:
  - *Duplicate, for a total of 20 bottles
  - *Batch culture method
  - *Hot shaker water bath (39^0C)at 60 shakes per minute for 24 hours

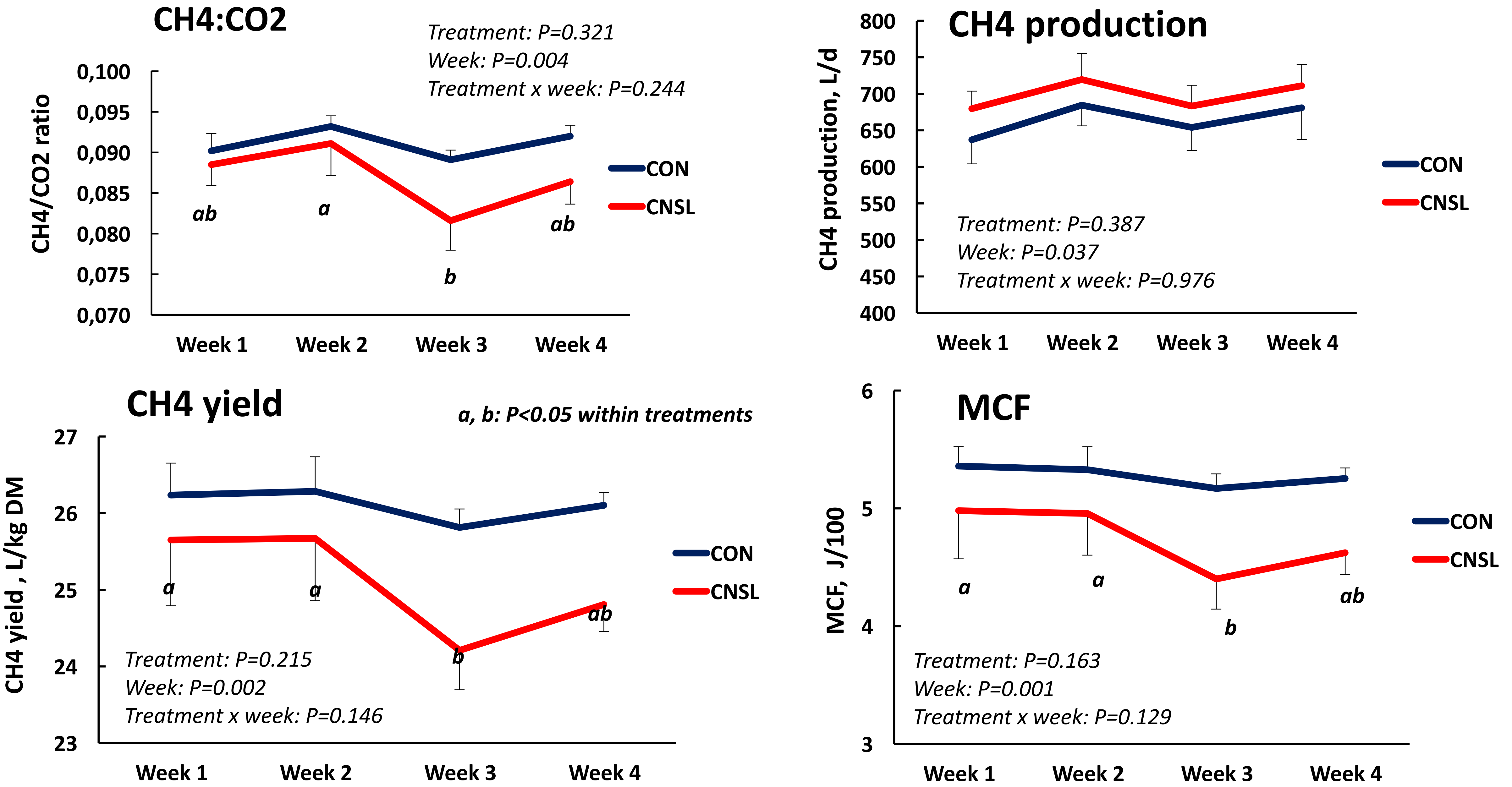
Results

Dry matter intake and milk yield

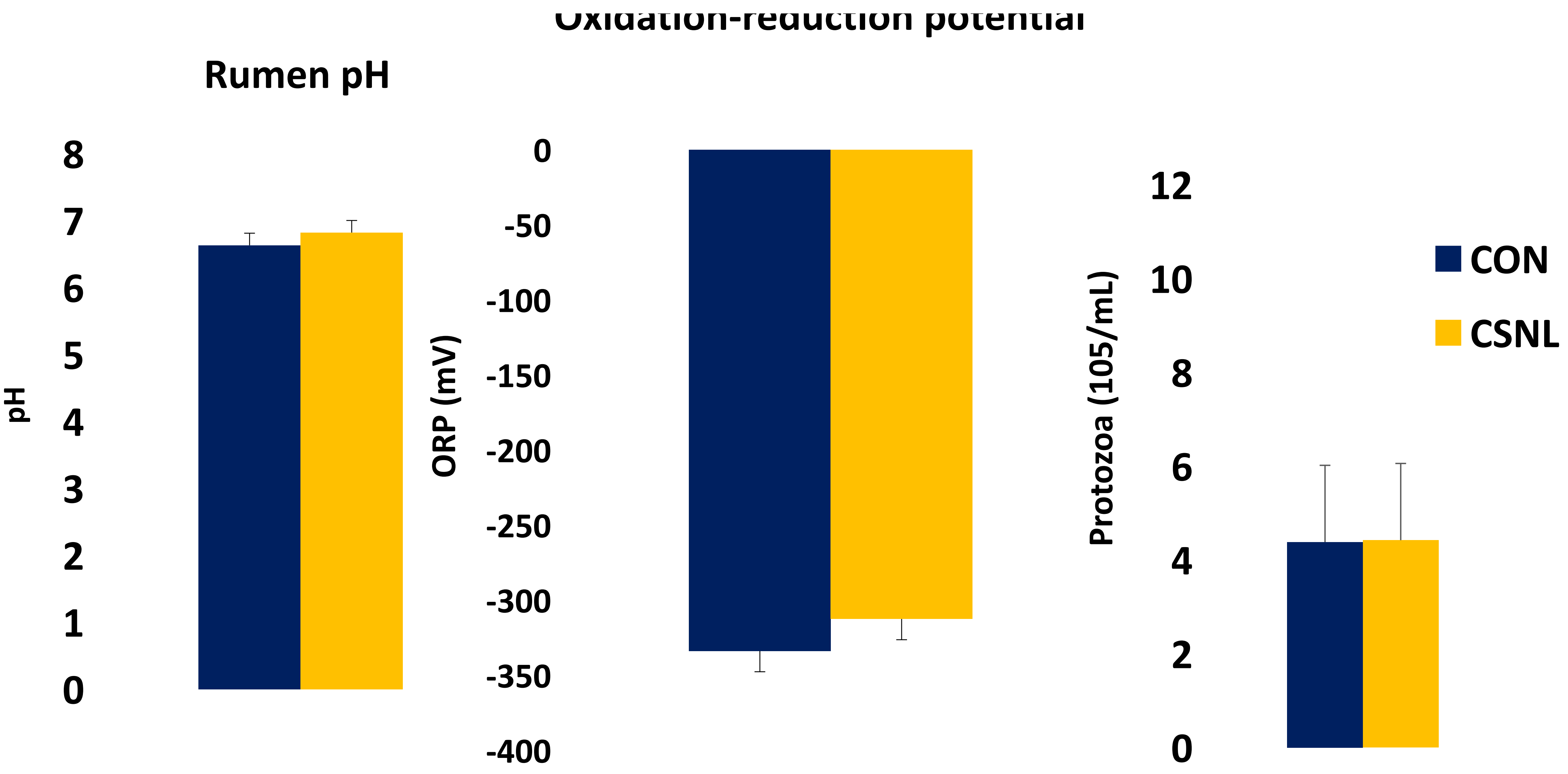


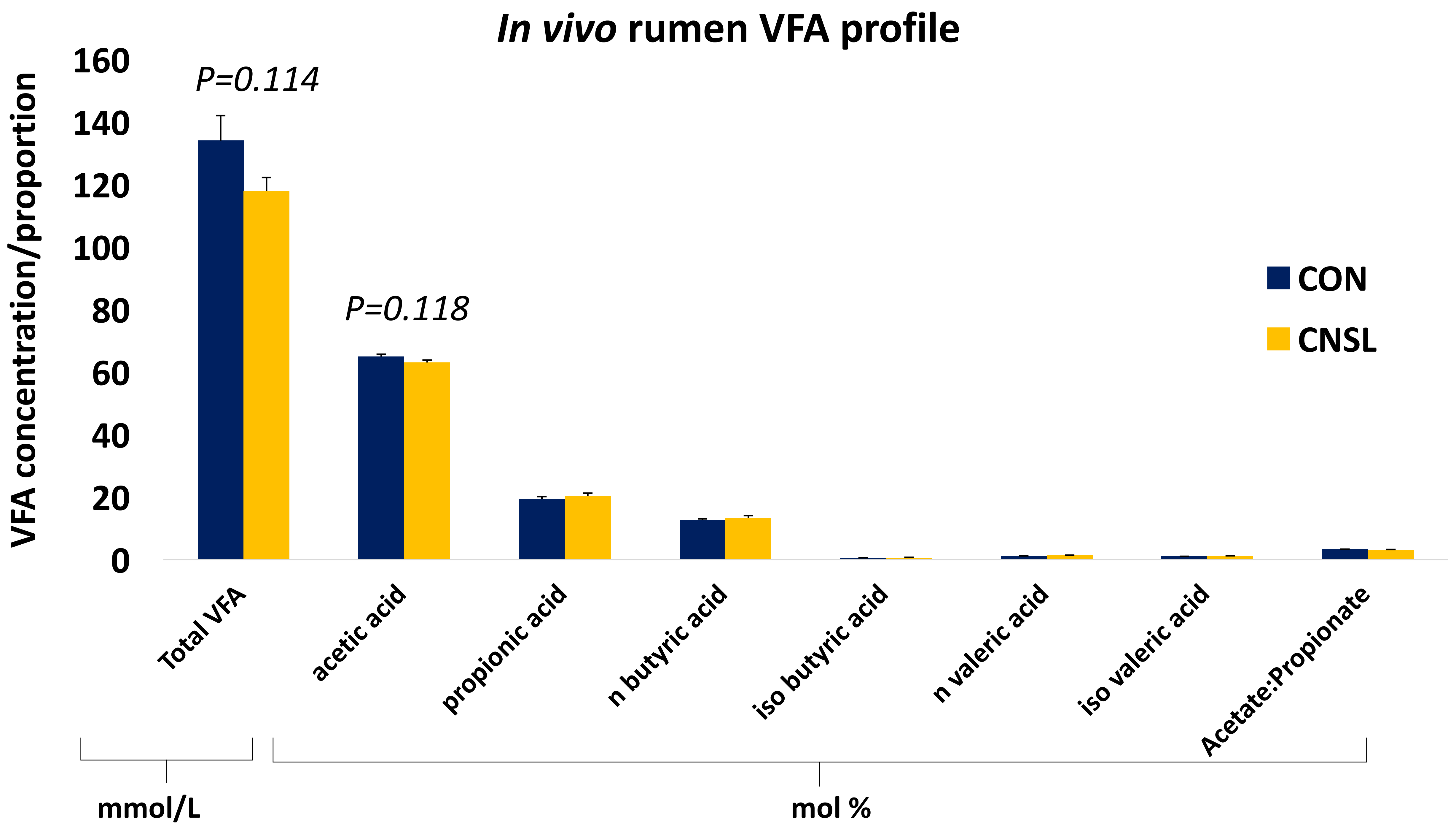


In vivo methane production



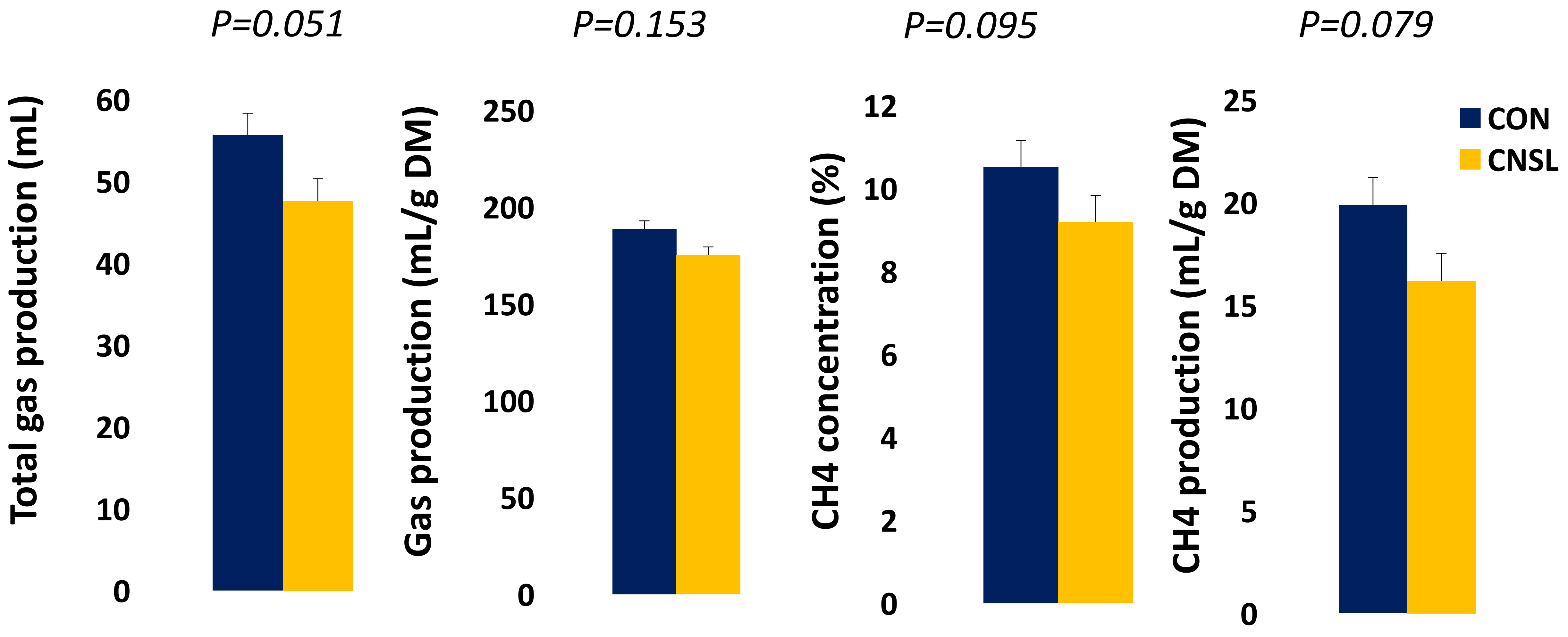
In vivo rumen profile

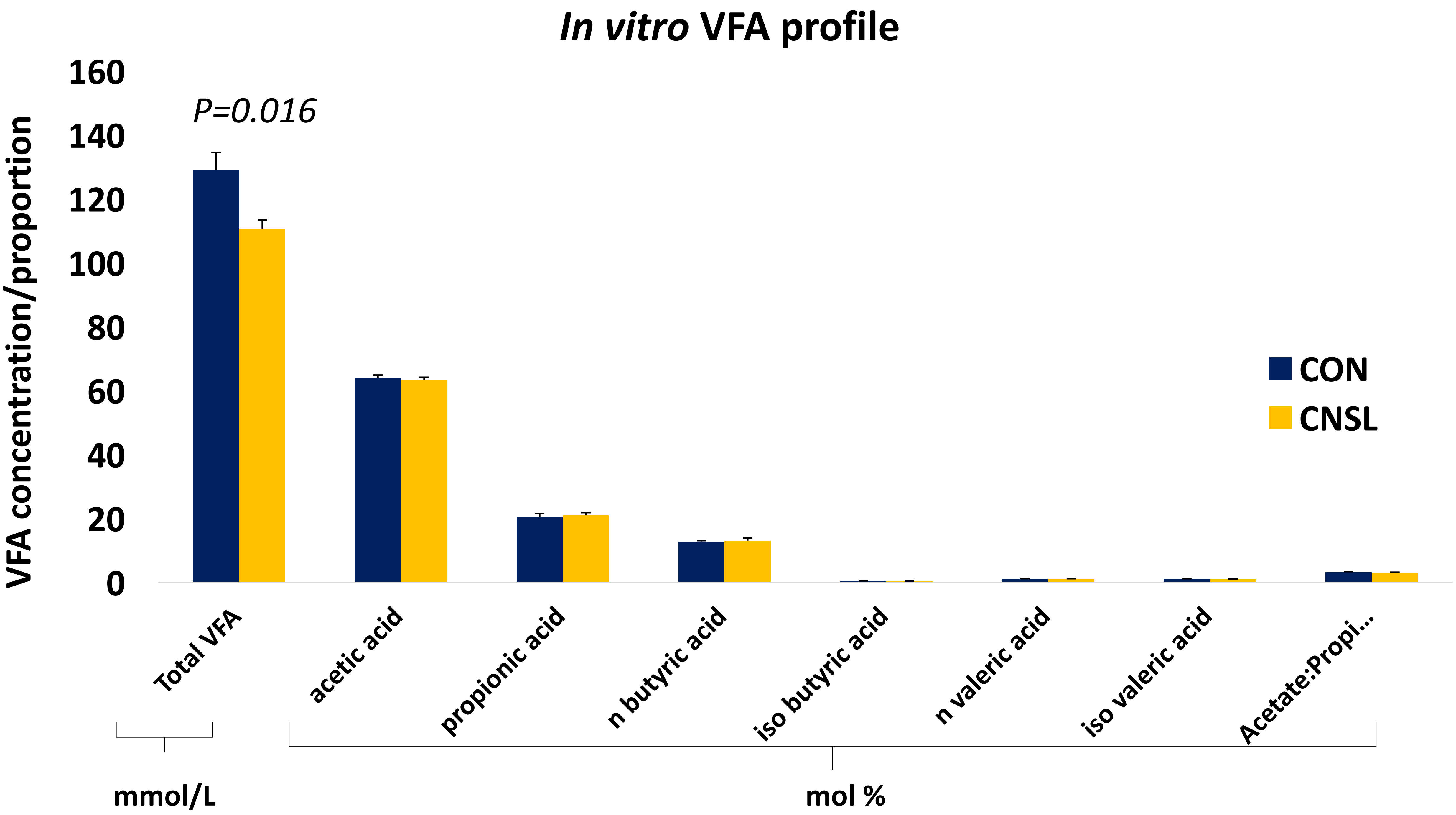


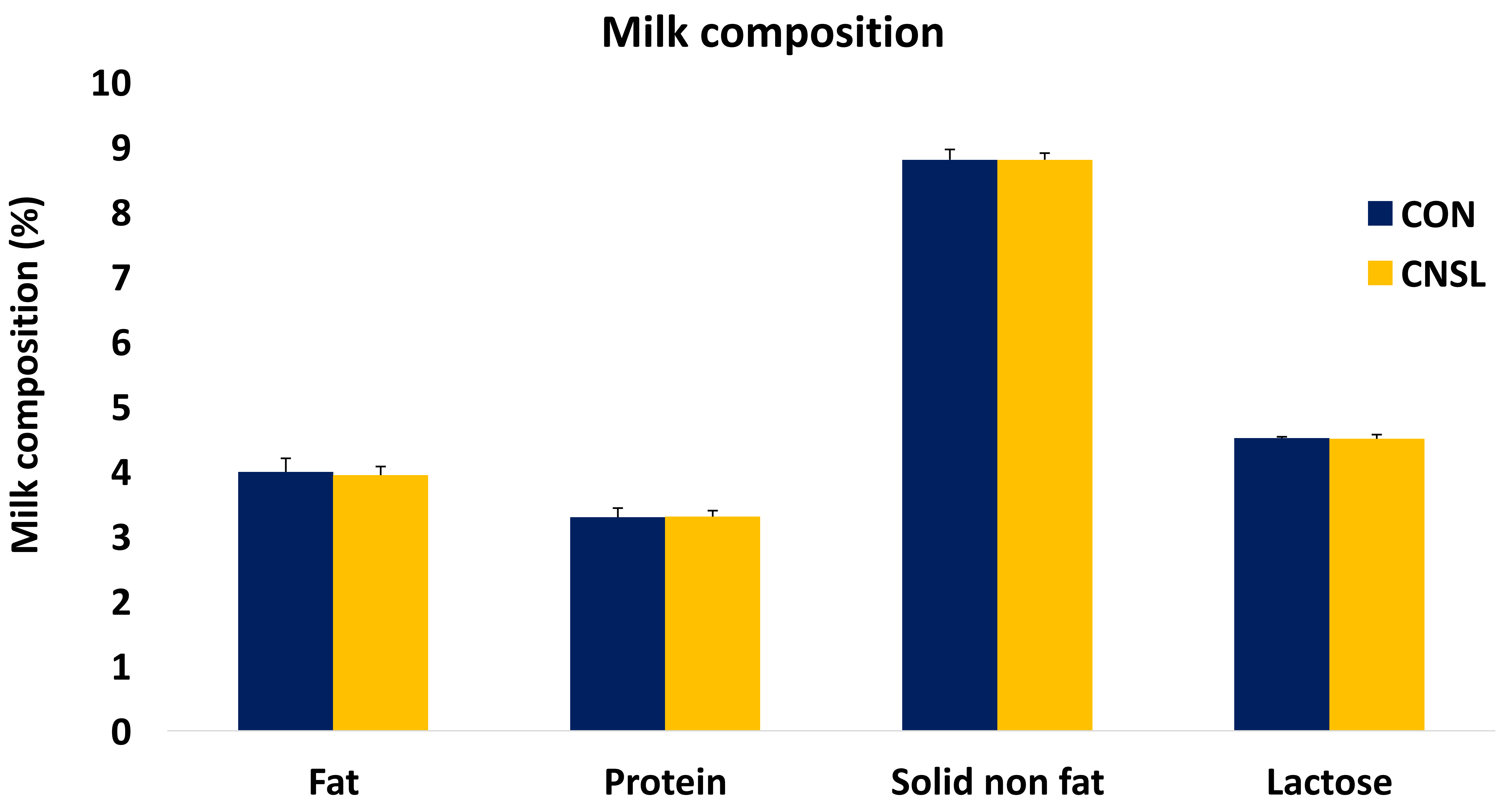


In vivo rumen VFA profile
VFA concentration/proportion
160
140
120
100
80
60
40
20
0
P=0.114
P=0.118
CON
CNSL
Total VFA
acetic acid
propionic acid
n butyric acid
iso butyric acid
n valeric acid
iso valeric acid
Acetate:Propionate
mmol/L
mol %

In vitro methane production




In vitro VFA profile
160
140
120
100
80
60
40
20
0
P=0.016
VFA concentration/proportion
CON
CNSL
Total VFA
acetic acid
propionic acid
n butyric acid
iso butyric acid
n valeric acid
iso valeric acid
Acetate:Propi...
mmol/L
mol %


Milk composition
Milk composition (%)
10
9
8
7
6
5
4
3
2
1
0
Fat
Protein
Solid non fat
Lactose
CON
CNSL

SUMMARY

- The addition of CNSL up to 10 g/day in the dairy cows' diet under a free stall barn tends to alter rumen fermentation and reduce CH_4 yield, without a negative effect on milk production and composition.
- In vitro, rumen fluid from CNSL cows tended to reduce total gas, methane, and VFA production compared to rumen fluid obtained from control cows.
- The sniffer method is a helpful way to detect the mitigating effect of CNSL in a free-stall barn.

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Thank you for listening