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In vitro incubation of dairy cow diets:

1. Degradability, total gas and methane yield

Maccarana L.¹, Cattani M.¹, Tagliapietra F.²,
Schiavon S.², Hansen H.H.³, Bailoni L.¹

¹Department of Comparative Biomedicine and Food Science, BCA (Italy)

²Department of Agronomy, Food, Natural resources, Animals and Environment, DAFNAE (Italy)

³Department of Large Animal Science (Denmark)



Methane emissions

- ✓ CH_4 is one of fermentation gases (CO_2 , CH_4 and VFA) → loss of gross energy intake to the animal, quantified around 2-12% (Johnson and Johnson, 1995)
- ✓ CH_4 is also considered an atmospheric greenhouse gas (GHG: CO_2 , CH_4 , N_2O , and O_3) → greenhouse effect & climate changes
- ✓ Assessments of CH_4 emissions vary considerably depending on the references and countries.

Objectives

- ✓ To evaluate *in vitro*
 - ~ Degradability
 - ~ Total gas production
 - ~ Methane yield

of diets for dairy cows actually used in the Veneto region, in order to estimate their range of variation on methane emission

Diets

- ✓ A **Reference Diet (RD)** → representative of dairy cow diets commonly used in the Veneto region
- ✓ Other 7 diets were formulated changing the proportions of CP, NDF and lipids, within the limits of viable:
 - ~ **Low Protein (LP)**
 - ~ **High Protein (HP)**
 - ~ **Low Fibre (LF)**
 - ~ **High Fibre (HF)**
 - ~ **Low Lipid (LL)**
 - ~ **High Lipid, fat supplement (HLF)** (calcium soaps of palm)
 - ~ **High Lipid, extruded oilseeds (HLO)**

Chemical composition (g/kg DM) of 8 diets

| | RD | LP | HP | LF | HF | LL | HLF | HLO |
|------------------------------|------|------|------|------|------|------|------|------|
| CP | 152 | 117 | 187 | 152 | 152 | 152 | 152 | 152 |
| NDF | 370 | 370 | 370 | 331 | 448 | 370 | 370 | 370 |
| ADF | 186 | 177 | 200 | 149 | 255 | 186 | 189 | 192 |
| ADL | 28 | 26 | 33 | 20 | 43 | 28 | 28 | 28 |
| Lipids | 33 | 33 | 33 | 33 | 33 | 22 | 55 | 55 |
| Starch | 271 | 333 | 203 | 329 | 151 | 282 | 246 | 231 |
| NFC | 393 | 434 | 350 | 438 | 307 | 403 | 372 | 371 |
| Ash | 52 | 46 | 60 | 46 | 60 | 52 | 52 | 52 |
| GE, MJ/Kg DM | 18.6 | 18.5 | 18.7 | 18.7 | 18.7 | 18.4 | 19.1 | 19.1 |
| ME _{TDN} , MJ/kg DM | 12.1 | 12.2 | 11.8 | 12.7 | 11.1 | 11.8 | 12.7 | 12.7 |

- ✓ Low Protein (LP) and High Protein (HP)
- ✓ Low Fibre (LF) and High Fibre (HF)
- ✓ Low Lipid (LL) and High Lipid: fat supplement: calcium soaps of palm (HLF) and High Lipid: extruded oilseeds (HLO)

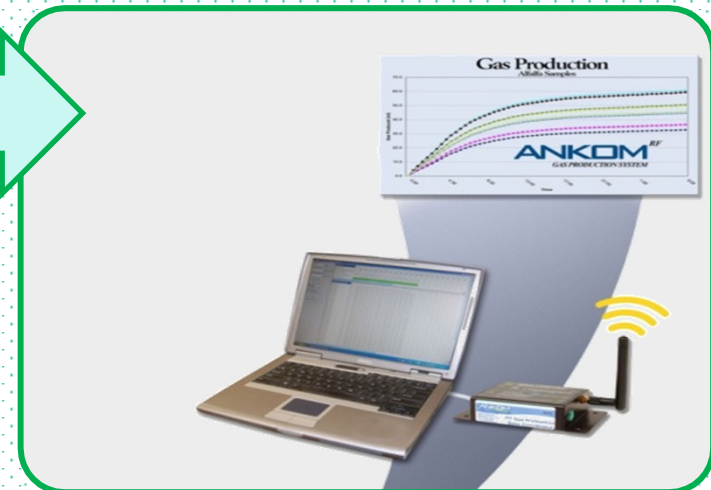
Experimental design

| | |
|-------------------------------|-----|
| Diets | 8 |
| Incubation times | 2 |
| Runs for each incubation time | 2 |
| Replications of diets for run | 5 |
| Total bottles | 160 |
| Blanks | 20 |

| | |
|-----------------|-------------|
| Temperature, °C | 39.0 |
| Feed sample, g | 1.0 ± 0.010 |
| Buffer, mL | 100 |
| Rumen fluid, mL | 50 |

Automated GP system: Ankom® RF

- ✓ Kit of 50 bottles (317 ml), with spout and screw cap with septum puncture-proof, equipped with:
 - ~ a pressure detector
 - ~ an open-closed valve for gas venting at 6.8 KPa
- ✓ All bottles are wireless connected to a PC
- ✓ Pressure values inside the bottles are recorded by PC every minute



Gas samples collection

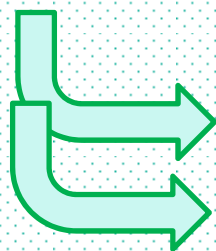
- ✓ 9 mL of fermentation gas were collected with a gas-tight syringe and injected into a Vacuette®
- ✓ An aliquot of 10 μL was analyzed for CH_4 by GC

MATERIAL & METHOD



Computations

- ✓ NDF (**NDFd, %**) and true dry matter (**TDMd, %**) degradability (Goering and Van Soest, 1970)
- ✓ Gas production (**GP, mL/g DM**) was calculated by a conversion of psi into mL (Lopez *et al.*, 2007)
- ✓ **Total CH₄ production** = CH₄ measured in bottle headspace × [(bottle headspace (257 mL) + GP mL)]
CH₄ loss was calculated according to Ramin and Huhtanen (2012)



mL/g DM incubated

mL/g TDM degraded

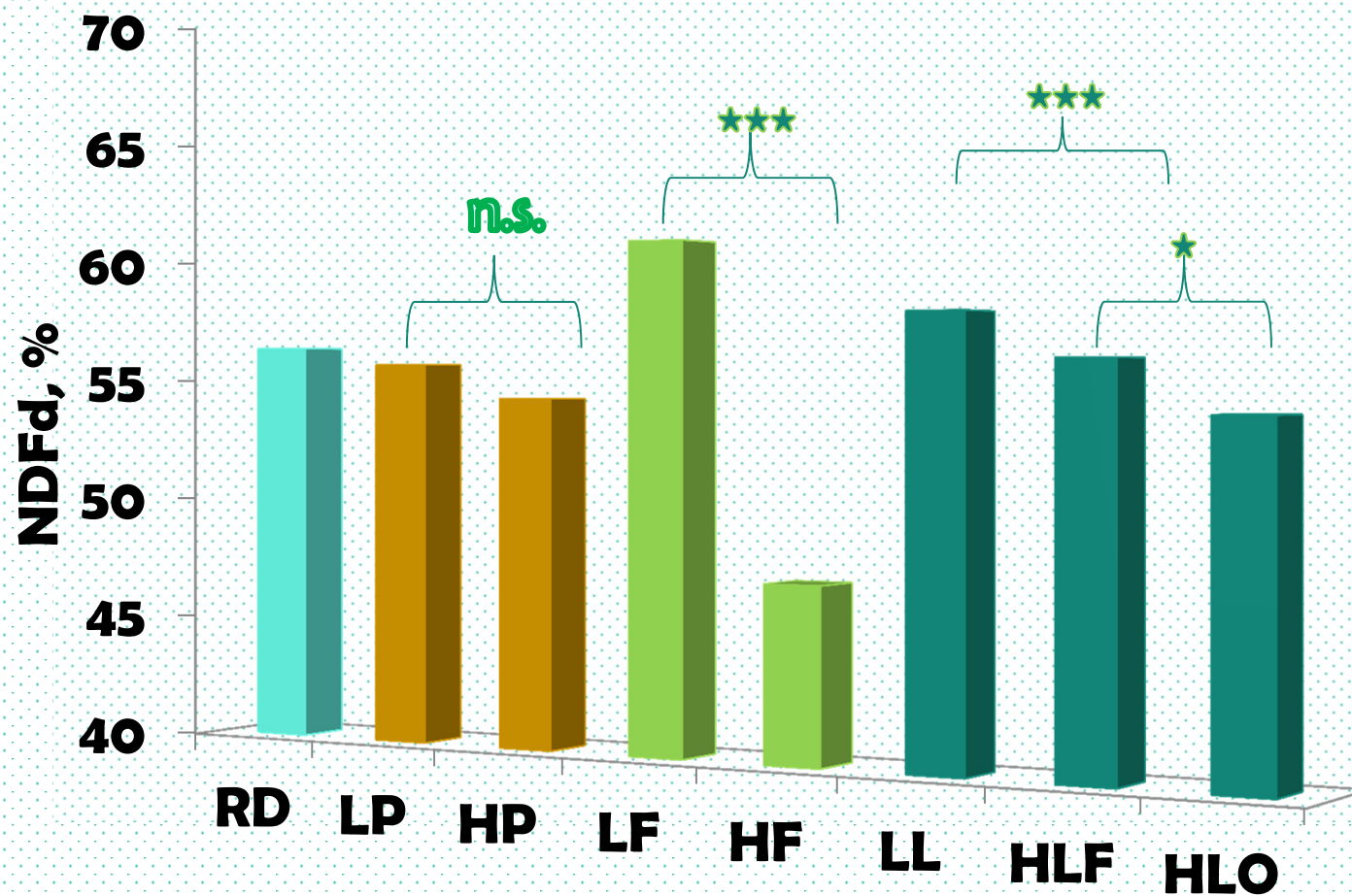
Statistical analysis

- ✓ ANOVA (PROC GLM, SAS):
 - ~ Diet (1-8)
 - ~ Incubation time (24 and 48 h)
 - ~ Run within incubation time (1-2)
- ✓ Contrasts:
 - ~ LP *vs* HP diet → effect of CP level
 - ~ LF *vs* HF diet → effect of NDF level
 - ~ LL *vs* (HFL + HOL)/2 → effect of lipid level
 - ~ HLF *vs* HLO → effect of lipid source

Effect of diet on NDFd (% NDF)

*** $P < 0.001$

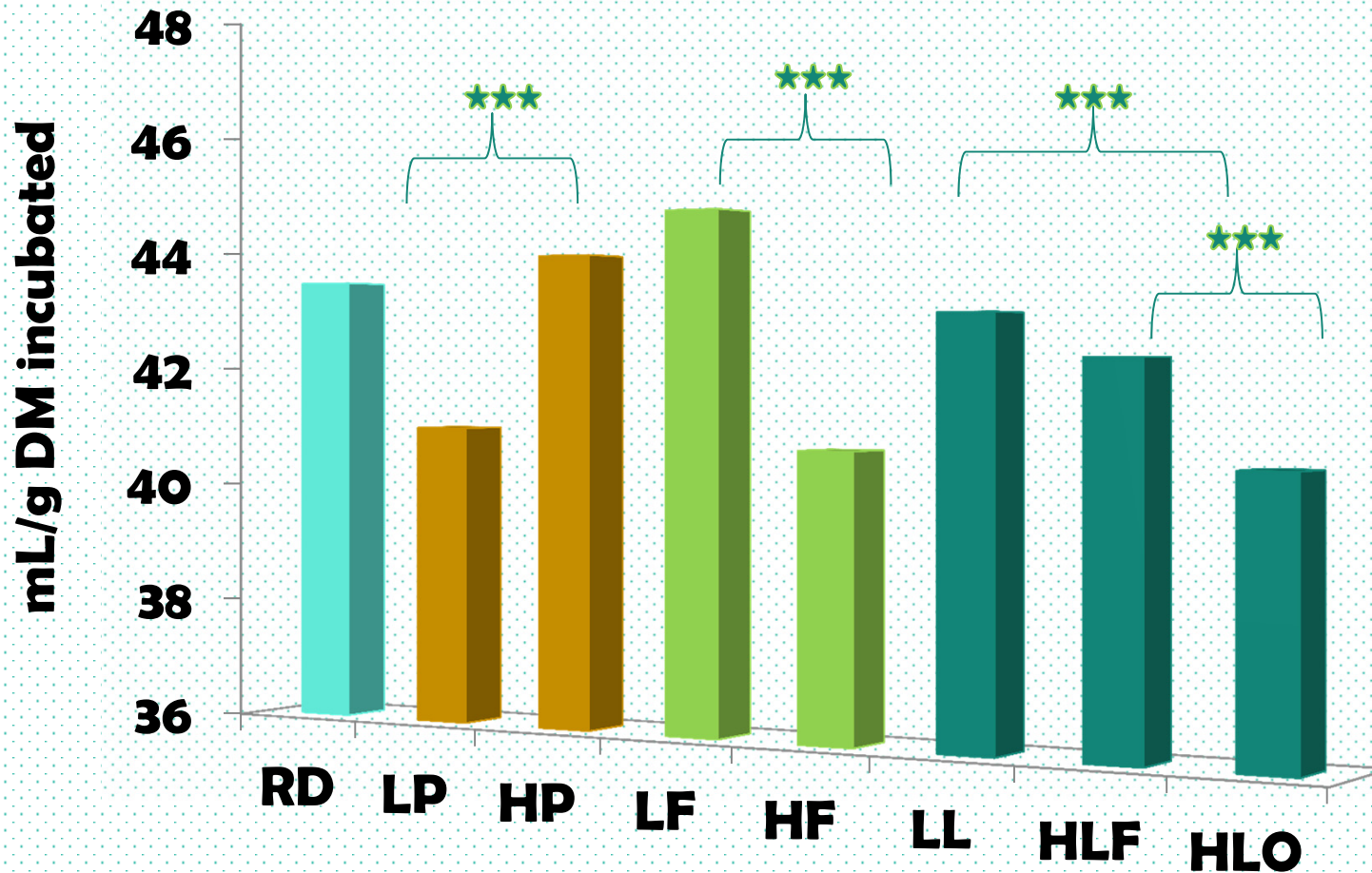
* $P < 0.05$



RESULTS &
DISCUSSION

Effect of diet on CH₄ (mL/g DM incubated)

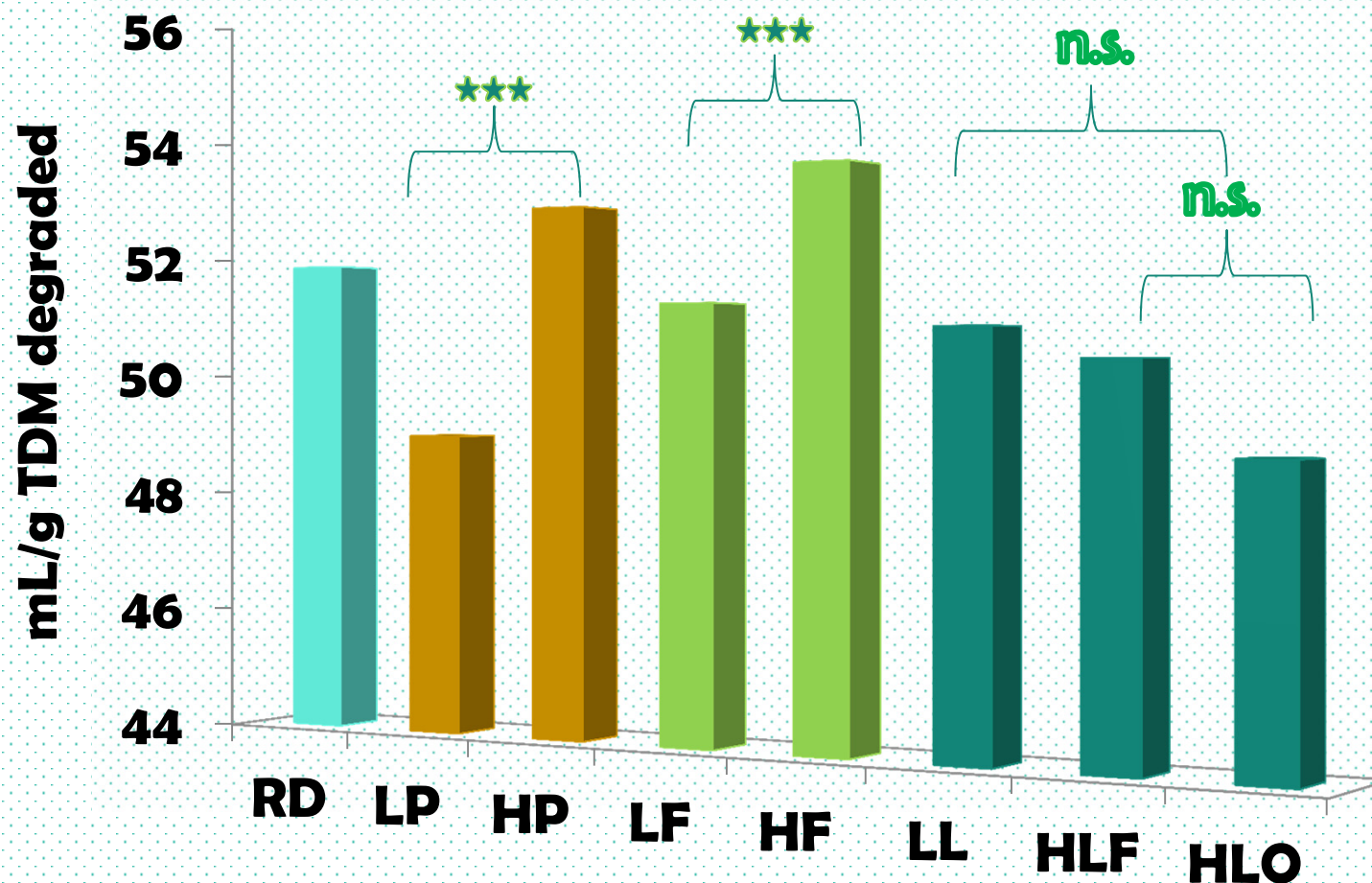
***P<0.001



RESULTS &
DISCUSSION

Effect of diet on CH₄ (mL/g TDM degraded)

***P<0.001



RESULTS &
DISCUSSION

Take home messages

- ✓ The amount of *in vitro* CH₄ produced was comparable to that reported by the literature
- ✓ The effects due to the CP, NDF and lipids were significant and comparable to other studies
- ✓ The magnitude of these effects is small in actual diets for lactating dairy cows
- ✓ In addition to N excretion, **low-protein diets** should also reduce CH₄ emission without negative effects on degradability of diet
This effect should be more carefully considered *in vivo*

Acknowledgements

- ✓ The present study was financed by “ARCHAEA” Project: “Feeding strategies to reduce methane emissions from dairy cows”, Veneto Region Rural Development Programme (RDP) 2007-2013



**THANKS FOR YOUR
ATTENTION**

FOR MORE INFORMATION:
laura.maccarana@studenti.unipd.it



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Ingredients (g/kg DM) of 8 diets

| | RF | LP | HP | LF | HF | LL | HLF | HLO |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Corn silage | 351 | 375 | 281 | 430 | - | 351 | 351 | 351 |
| Alfalfa hay | 89 | 66 | 156 | 23 | 134 | 89 | 89 | 89 |
| Ryegrass hay | 47 | 43 | 52 | - | 231 | 47 | 52 | 56 |
| Meadow hay | 47 | 47 | 52 | - | 227 | 47 | 52 | 60 |
| Corn meal | 205 | 258 | 147 | 228 | 152 | 218 | 182 | 160 |
| Barley meal | 119 | 160 | 90 | 171 | 92 | 122 | 100 | 100 |
| Soybean meal | 113 | 27 | 188 | 119 | 126 | 126 | 121 | 18 |
| Whole soybean | - | - | - | - | - | - | - | 68 |
| Extruded soybean | - | - | - | - | - | - | - | 68 |
| Extruded flaxseed | 29 | 24 | 34 | 29 | 38 | - | 29 | 29 |
| Fat supplement | - | - | - | - | - | - | 24 | - |

Incubation procedures

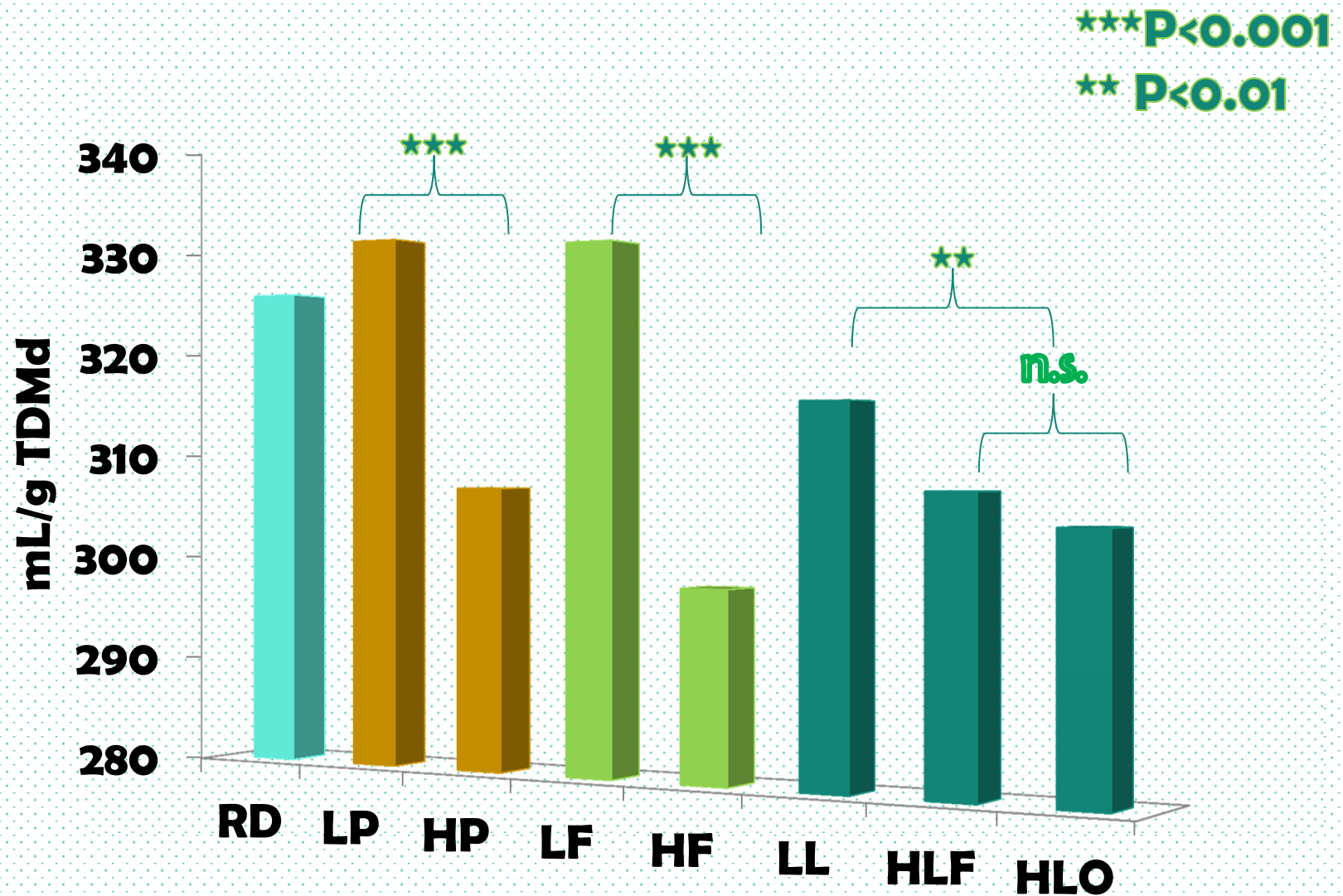
- ✓ Rumen fluid was collected by an esophageal probe
- ✓ Buffer solution was prepared according to Menke and Steingass (1988)



Gas chromatograph

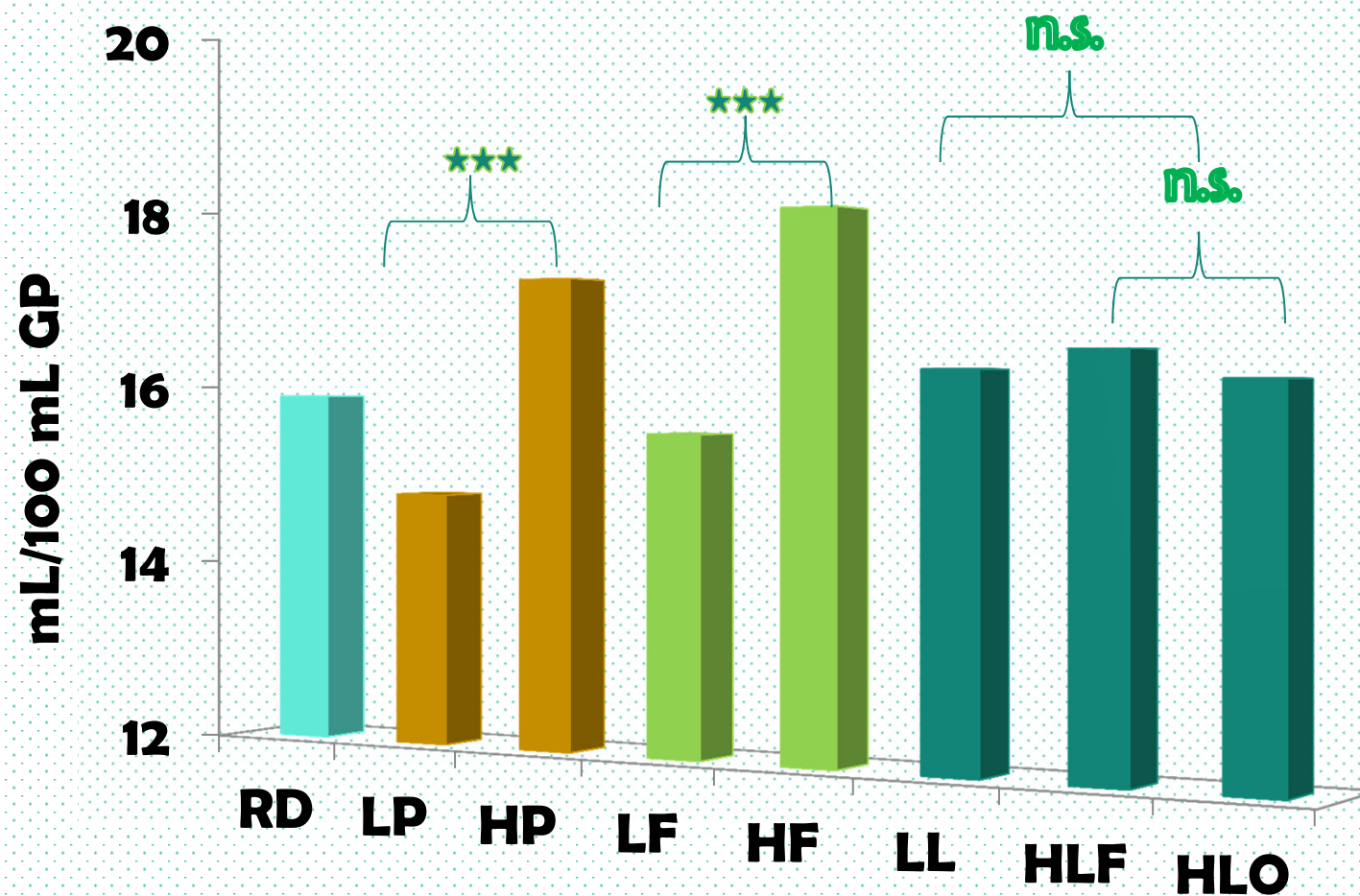
- ✓ Model 7820A GC system, Agilent Technologies
- ✓ Flame ionization detector
- ✓ 30 m stainless steel column, GS-CarbonPLOT, Agilent Technologies
- ✓ Gas carrier: Hydrogen, flow rate: 1.6 ml/min
- ✓ Isothermal oven temperature: 40°C

Effect of diet on GP (mL/g TDMd)



Effect of diet on CH₄ (mL/100 mL GP)

***P<0.001



Effect of variation in CP, NDF and lipid content on CH₄ reduction

✓ A decrease of 1% of chemical compound in the diet:

| | mL CH ₄ / g TDMd | mL CH ₄ / g DM | <i>KJ CH₄ / MJ GE</i> | Ramin and Huhtanen (2013) In vivo KJ CH ₄ / MJ GE |
|-------|--------------------------------|------------------------------|--------------------------------------|--|
| CP | -0.55 | -0.41 | -0.79 | n.s. |
| NDF | -0.21 | +0.33 | +0.63 | -0.46 |
| Lipid | +0.55 | +0.45 | +0.86 | +1.30 |