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The aim of this work was to study the impact of a crude protein (CP) restriction on productive performance, metagenomics and metabolic profile in growing (120 to 270 days of age) Holstein calves intensively reared. Forty calves were assigned to two dietary treatments: CP in the concentrate was formulated either based on the levels used commercially (CTR: 12% CP on an as-fed basis) or reducing them (LP: 10% CP on an as-fed basis). Concentrate was supplemented with barley straw and both were supplied *ad libitum*. Live weight (LW) and concentrate intake were registered automatically. Ten animals per treatment (220 kg of LW and 155 days of age) were sampled to determine nitrogen balance, rumen metagenome, and urinary/plasma metabolic profiles. Nitrogen balance was estimated by difference between intake and excretion (urinary plus faecal excretions). Rumen bacterial and archaeal community composition was analysed by taxonomic profiling of 16S ribosomal RNA variable regions. Urine and plasma samples were analysed by liquid chromatography coupled to mass spectrometry. The results showed that, at the beginning of the growing phase, CP restriction reduced average daily gain in LP animals (1.65±0.04 vs 1.77±0.04 kg/d, for LP and CTR respectively, P=0.044). Nitrogen excretion (65.9±5.3 vs 81.7±5.3 g/d, for LP and CTR respectively, P=0.049) and retention (30.2±4.2 vs 48.7±4.2 g/d, for LP and CTR respectively, P=0.006) were lower in LP animals than in CTR animals. Only 69% of detected operational taxonomic units were common between both treatments and protein restriction raised richness levels in rumen microbiota (129.9±7.8 vs 101.4±7.8 OTUs/animal, for LP and CTR respectively, P=0.019). Dietary CP restriction led to an increase in *Actinobacteria* phylum, mainly integrated by *Bifidobacterium* genera which is capable of fermenting starch. CP restriction induced the appearance of new discriminant metabolites, being this effect clearer in urine than plasma samples.

Genetic and environmental influence on colostrum quality and absorption in Swedish dairy cattle

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Colostrum with sufficient IgG content is essential for the newborn calf, as it requires this passive immunity to survive until weaning. Previous studies have shown a high variation in the amount of colostrum antibodies in dairy cows, with a large proportion having low antibody levels. Failure of passive transfer (FPT) occurs when a calf does not absorb enough antibodies (<10 g/l of IgG in serum) from the colostrum. Some calves absorb antibodies very effectively while others do not. This difference in uptake cannot be explained solely by the time, amount and quality of the colostrum given. The purpose of this study is to identify genetic and environmental factors that can explain this difference in the effectiveness of antibody uptake in calves and variation in colostrum quality in cows. Three experimental farms were included in the study. Colostrum samples from 1,311 cows calving from January 2015 to April 2017 were collected and analysed by Brix refractometer to estimate antibody concentration. For two of the farms, serum from 785 calves was collected at 2 to 7 days after birth and analysed by total IgG ELISA. Brix values ranged from 6.5 to 38.9%, and calves serum IgG from 1.1 to 91.6 g/l. Preliminary results using a linear mixed model show an effect of breed on colostrum quality with Holstein cows displaying significantly higher values than Swedish Red ($P=5\times10^{-12}$). This was also true for samples with shorter calving to colostrum sampling times ($P=2\times10^{-16}$). Genetic parameters will be estimated for colostrum Brix values, serum total IgG and Apparent Efficiency of Absorption (AEA). Early estimates of Herd-Year-Season explain 2.5 to 16% of the variation observed.

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Effects of protein reduction on performance, rumen metagenome and metabolic profile in Holstein calves

Costa, S.; Balcells, J.; de la Fuente, G., Mora, J.; Álvarez, J.; Villalba, D.

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MINISTERIO DE AGRICULTURA Y PESCA, ALIMENTACIÓN Y MEDIO AMBIENTE

INTRODUCTION

- High concentrate diets for beef calves in the Mediterranean region (90% concentrate: 10% forage).
- Current CP recommendations: 16-18% (DM) for growing calves.

INTRODUCTION

- Some studies obtained similar ADG and less N waste when reducing CP content to 12-14% (DM).

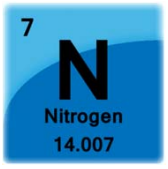
POSSIBLE OVERESTIMATION

- Adaptation due to: animal's metabolism or rumen microbiota.

CONTROVERSIAL RESULTS

OBJECTIVE

- Study the effect of reducing CP supply from 14% to 12% (DM) in growing calves.
- Assessing its impact on:
  - Performance.
  - Nitrogen balance.
  - Rumen microbiota.
  - Metabolic profile.



MATERIAL AND METHODS

- Twenty male Holstein calves (118±1 days of age and 162±5 kg of body weight).
- Two experimental treatments:
  - CTR: Commercial concentrate (CP: 14% [DM]) plus barley straw.
  - LP: Low protein concentrate (CP: 12% [DM]) plus barley straw.

***AD LIBITUM* FED**
FREE ACCESS TO WATER

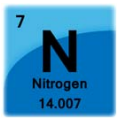
MATERIAL AND METHODS

- Live weight and concentrate intake automatically registered throughout the experimental period.

MATERIAL AND METHODS

- Sampling in week 6 of experimental period:
  - Nitrogen balance:

    N retention = N intake – N urine – N faeces



  - Rumen microbial community:

    Taxonomic profiling of 16S ribosomal RNA

  - Metabolic profile:

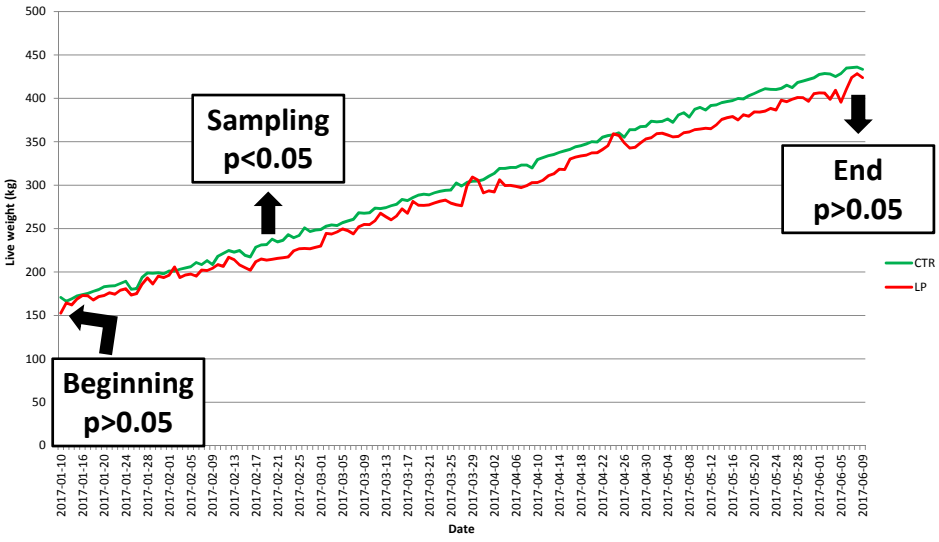
    All metabolites and plasma and urine

RESULTS: PERFORMANCE

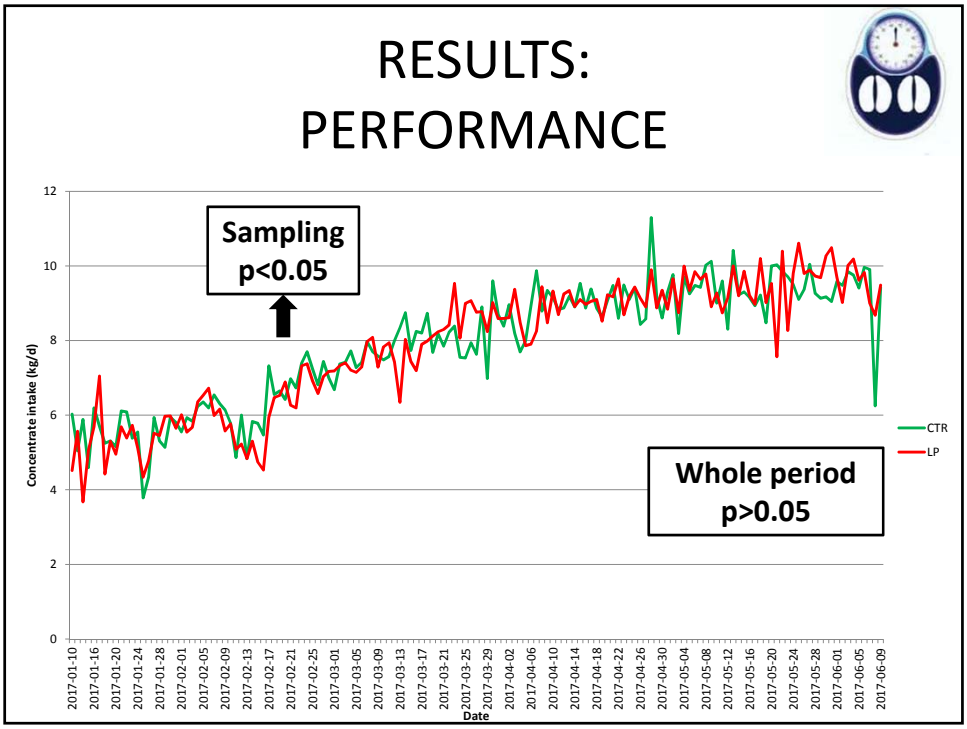
	CTR	LP	SE
n	10	10	
Live weight (kg)	234.7[a]	214.8[b]	6.00
ADG (kg/d)	1.8	1.5	0.06
Concentrate intake (kg/d)	6.6[a]	5.7[b]	0.22
Concentrate conversion index	3.7	3.7	0.14

Week 6 Sampling

RESULTS: PERFORMANCE

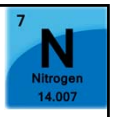


RESULTS: PERFORMANCE



Week 6 Sampling

RESULTS: NITORGEN BALANCE



	CTR	LP	SE
n	10	10	
N intake (g/d)	152.3[a]	113.8[b]	4.52
N excreted in urine (g/d)	28.7	18.7	4.16
N excreted in faeces (g/d)	54.5[a]	45.8[b]	1.82
N retention (g/d)	69.0[a]	49.2[b]	4.73
N retention (% N intake)	45.5	43.3	3.12

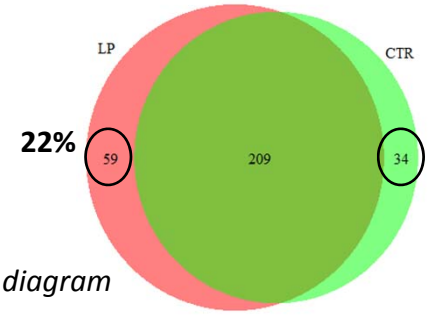
Week 6 Sampling

RESULTS: RUMEN MICROBIOTA

	CTR	LP	SE
n	10	10	
NH_3-N (mg/L)	4.15[a]	0.58[b]	1.185
pH	7.29	7.28	0.126
VFA (mmol/L)	66.96	57.81	8.512
Acetate (%)	46.30	47.79	0.887
Propionate (%)	43.59[a]	39.46[b]	0.734
Butyrate (%)	6.42[b]	9.27[a]	0.198

Week 6 Sampling

RESULTS: RUMEN MICROBIOTA



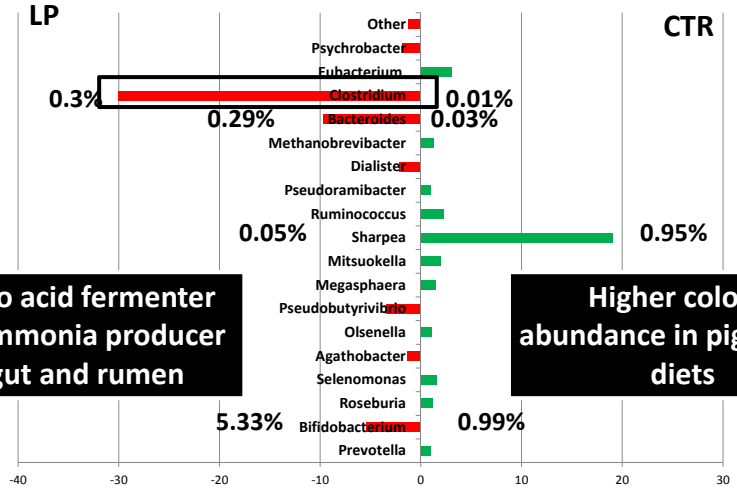
Venn diagram

Protein intake reduction raised rumen microbial diversity levels

	CTR	LP	SE
n	10	10	
Shannon Index	1.0[b]	1.7[a]	0.12
Simpson Index	0.3[b]	0.6[a]	0.04
Richness (OTUs/animal)	101.4[b]	129.9[a]	7.78

Week 6 Sampling

RESULTS: RUMEN MICROBIOTA



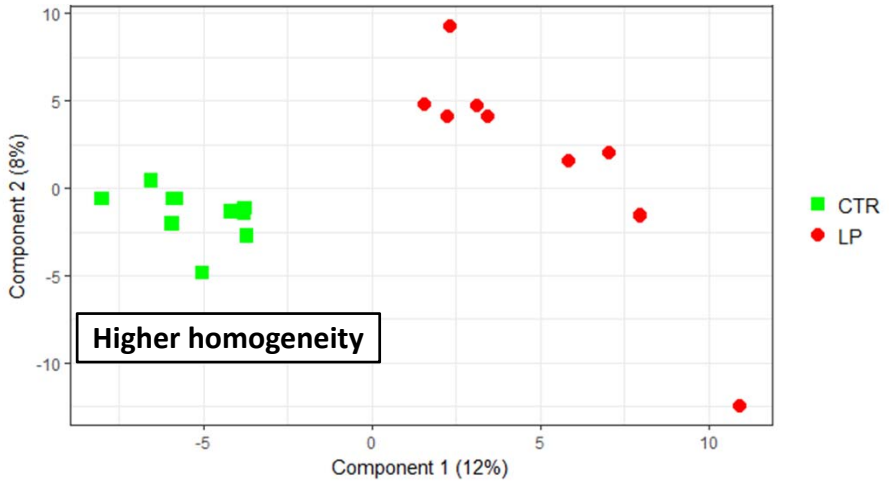
Amino acid fermenter and ammonia producer in gut and rumen

Higher colonic abundance in pigs fed LP diets

Fold change in genera abundance

Week 6 Sampling

RESULTS: RUMEN MICROBIOTA



Rumen microbiota composition was modified by experimental treatment

Partial least-squares discriminant analysis

Week 6 Sampling

RESULTS: METABOLIC PROFILE

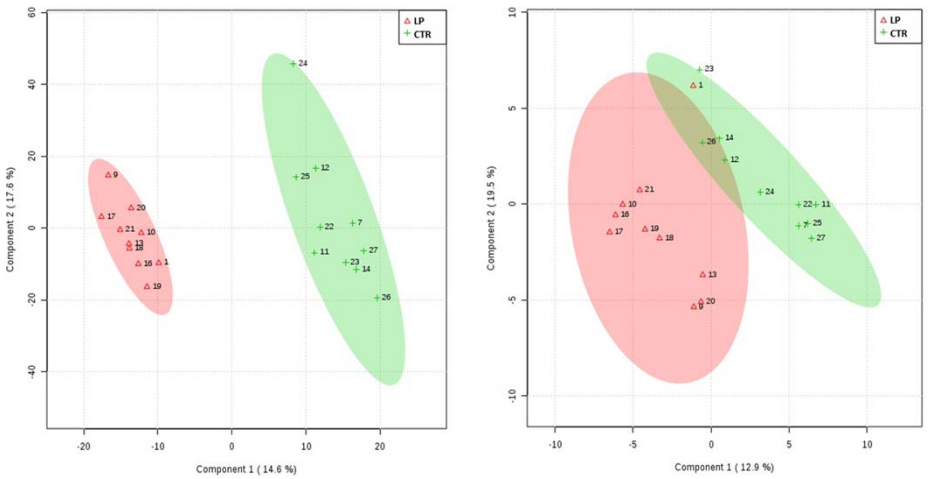
	Total detected metabolites	Discriminant metabolites
n	10	10
Urine (negative ionization)	1,555	139
Urine (positive ionization)	1,075	120
Plasma (negative ionization)	171	31
Plasma (positive ionization)	412	20
TOTAL	**3,213**	**310**

Protein reduction effect?

Week 6 Sampling

RESULTS: METABOLIC PROFILE

Urine **Plasma**



Partial least-squares discriminant analysis

Week 6 Sampling

CONCLUSIONS

- Dietary protein reduction led to:
  - Lower dry matter intake and performance.
  - Lower nitrogen retention and waste.
  - Higher ruminal microbiota biodiversity.

Similar performance results in the entire experimental period

Evidence of rumen microbiota adaptation to a low protein diet?

  - New metabolites appearance.

Different metabolic pathways to cope with a low protein diet?

THANK YOU FOR YOUR ATTENTION!

Effects of protein restriction on rumen metagenome and metabolic profile in Holstein calves

Costa, S.; Balcells, J.; de la Fuente, G., Mora, J.; Álvarez, J.; Villalba, D.

Introduction

Nowadays animal production systems need to be redesigned, especially the most intensive ones (Dumont *et al.*, 2014). As a good example of intensive animal production system, beef cattle feedlot fattening faces different challenges: environmental impact minimization, use of feed fit for human consumption reduction and self-sufficiency increase. In this sense, dietary protein level optimization may have some benefits, being worth to highlight the decrease of animals' emission of pollutant compounds to soil (undigested N) and atmosphere (urinary excreted N metabolism products which are susceptible to converting and being emitted as ammonia and nitrous oxide) (McDonald *et al.*, 2010).

Objectives

Bearing in mind all previously said, the aim of this work was to study the impact of a crude protein restriction on productive performance, rumen metagenome and metabolic profile in growing (120-270 days of age) Holstein calves intensively reared. For this reason, the response of a dietary protein level decrease below the standards proposed by FEDNA (2008) was evaluated.

Materials and methods

Forty calves were assigned to two treatments: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP).

Live weight and concentrate intake were automatically registered in farm facilities. A sampling of ten animals per treatment was done (221 kg of body weight and 155 days of age) to determine nitrogen balance, rumen metagenome and metabolic profile. Nitrogen balance was estimated from nitrogen intake and nitrogen urinary and faecal excretions. Rumen bacterial and archaeal community composition was analysed by taxonomic profiling of 16S ribosomal RNA variable regions. Finally, metabolic profile was determined from urine and plasma samples by liquid chromatography coupled with mass spectrometry.

Data were analysed with the JMP® (2015) statistical software. Body weight, average daily gain, concentrate intake, nitrogen balance, microbial abundances and

microbial biodiversity data were analysed through analysis of variance, including treatment as the only effect with two levels (CTR and LP). Comparisons among treatments were performed by the Tukey's method.

Venn diagram and partial least squares discriminant analysis (PLSDA) figure were produced using R Core Team (2017) statistical software.

Results and discussion

The results showed that, at the beginning of the growing phase, crude protein restriction reduced average daily gain and, as a consequence, final average daily gain was lower in LP animals than in CTR animals. Animals of both treatments had the same concentrate intake but concentrate conversion index was lower in CTR animals (Table 1).

Table 1 Initial and final body weight, average daily gain, concentrate intake and concentrate conversion index per treatment in growing Holstein calves (168-410 kg of body weight and 120-270 days of age) receiving two different diets: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP). Standard error and treatment effect significance are also shown.

Item[1]	Treatment		Standard error	P-value
	CTR	LP		
n	20	20		
Initial body weight (kg)	168.60	166.50	3.95	0.512
Final body weight (kg)	418.80	400.30	8.37	0.127
Average daily gain (kg/d)	1.77[a]	1.65[b]	0.04	0.044
Concentrate intake (kg FM/d)	7.63	7.65	0.20	0.932
Concentrate conversion index	4.59[a]	4.92[b]	0.10	0.021

[1] FM: fresh matter.

LP animals consumed less nitrogen, as it was expected. Crude protein restriction led to a reduction of nitrogen faecal excretion and retention; however, nitrogen urinary loss remained unchanged between experimental groups (Table 2).

Table 2 Nitrogen intake, faecal and urinary excretion and retention per treatment in growing Holstein calves (221 kg of body weight and 155 days of age) receiving two different diets: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP). Standard error and treatment effect significance are also shown.

Item[1]	Treatment		Standard error	P-value
	CTR	LP		
n	10	10		
N intake (g/d)	130.45[a]	96.13[b]	3.29	<.001
N excretion (g/d)				
Faeces	53.04[a]	47.31[b]	1.77	0.035
Urine	28.67	18.61	4.16	0.104
N retention (g/d)	81.72[a]	65.92[b]	5.29	0.049

[1] N: nitrogen.

Operational taxonomic units' (OTUs) distribution is presented in a Venn diagram, which shows shared and not shared OTUs by animals of both treatments, depending of overlap (Figure 1). It displays that 209 OTUs were common between animals and experimental treatments, which represents the 69% of the total.

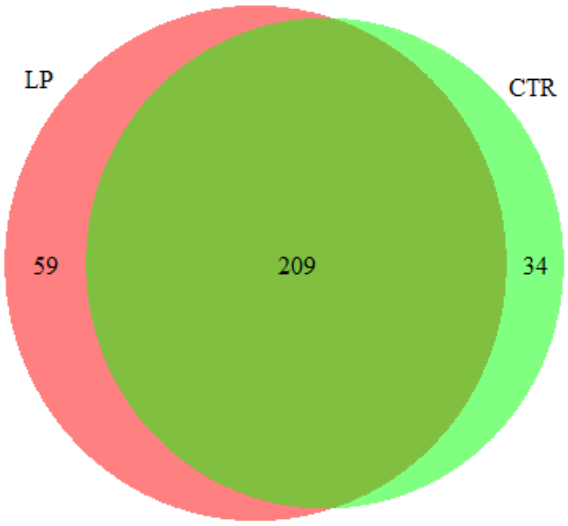


Figure 1 Venn diagram which shows shared and not shared OTUs by LP and CTR animals. Obtained in growing Holstein calves (221 kg of body weight and 155 days of age) receiving two different diets: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP).

Graphical representation of PLSDA of rumen microbial community is presented in Figure 2. CTR animals are represented by green squares and LP ones are represented by red dots. PLSDA plot is obtained from the first and second main components of the analysis, which are represented in the x-axis and y-axis, respectively. The percentage of the main components represent the relative contribution of this component to sample differences, which is a measure of the amount of original information extracted by this main component. The distance between the sample points represent the similarity of microbiota in the samples: samples that cluster together are composed of similar microbiota. PLSDA analysis revealed that rumen microbial community composition was modified by experimental treatment because animals with different level of protein intake are clearly separated in the ordination plot.

In general terms, crude protein restriction raised rumen microbial biodiversity levels (Table 3). More particularly, crude protein restriction led to an increase in *Actinobacteria* phylum, mainly integrated by *Bifidobacterium* and *Olsenella* genus. On the one hand, *Bifidobacterium*, which has been previously identified in rumen metagenome of animals consuming high concentrate diets, is capable of fermenting starch producing acetic and lactic acids (Hernandez *et al.,* 2008). On the other hand, bovine rumen is a likely habitat for *Olsenella*, which presents a marked proteolytic activity producing lactic acid as a final catabolite (Kraatz *et al.*, 2011).

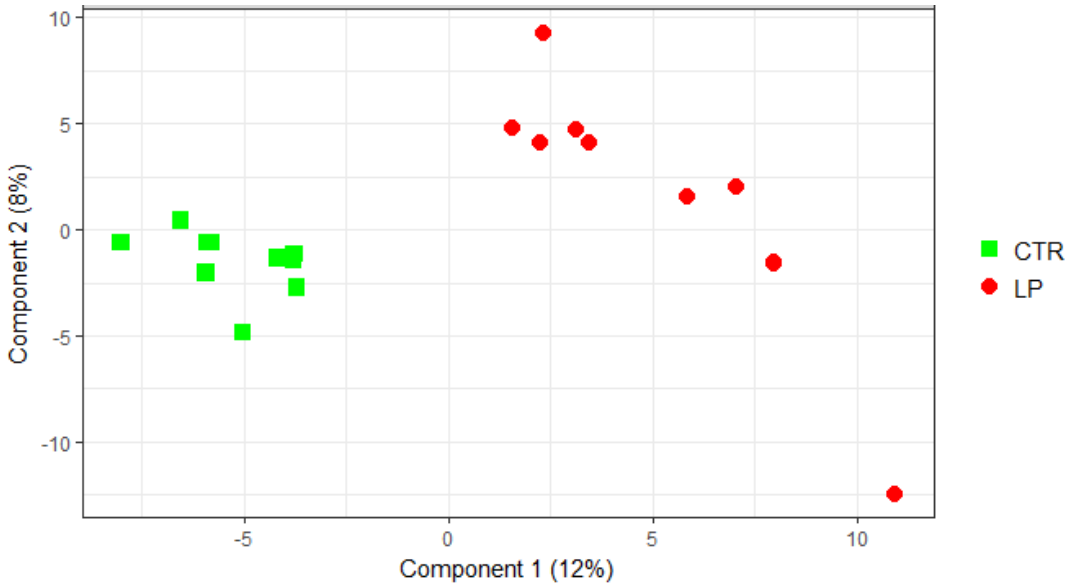


Figure 2 Partial least squares discriminant analysis (PLSDA) of rumen microbial community. Obtained in growing Holstein calves (221 kg of body weight and 155 days of age) receiving two different diets: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP).

Table 3 Main phylum and archaeal relative abundance (%) and microbial biodiversity indices per treatment in growing Holstein calves (221 kg of body weight and 155 days of age) receiving two different diets: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP). Standard error and treatment effect significance are also shown.

Item[1]	Treatment		Standard error	P-value
	CTR	LP		
n	10	10		
Phylum and archaeal relative abundance (%)				
Bacteroidetes	85.60	82.32	1.79	0.211
Firmicutes	11.48	10.70	1.35	0.687
Actinobacteria	2.37[b]	6.45[a]	1.02	0.011
Fibrobacteres	0.12	0.04	0.04	0.152
Proteobacteria	0.19	0.29	0.05	0.190
Spirochaetes	0.01	0.00	0.00	0.093
Tenericutes	0.03	0.04	0.01	0.698
Archaea	0.19	0.15	0.02	0.262
Microbial biodiversity indices				
Ratio F/B	0.14	0.13	0.02	0.862
Shannon index	1.03[b]	1.71[a]	0.12	0.001
Simpson index	0.34[b]	0.64[a]	0.04	<.001
Richness (nº OTUs/animal)	101.40[b]	129.90[a]	7.78	0.019

[1] F: *Firmicutes*; B: *Bacteroidetes*; OTUs: *Operational Taxonomic Units*

Above 3,000 metabolites were detected by metabolic profiling of urine and plasma samples (Table 4). New metabolites (around 300 out of the total), which were discriminant between experimental treatments, became present because of crude protein restriction; being this effect more clear in urine samples than in plasma ones.

Table 4 Total detected metabolites and discriminant metabolites between treatments in growing Holstein calves (221 kg of body weight and 155 days of age) receiving two different diets: commercial concentrate (12% crude protein on an as-fed basis) plus barley straw (CTR) or low protein concentrate (10% crude protein on an as-fed basis) plus barley straw (LP).

Item	Total detected metabolites	Discriminant metabolites
Urine (negative ionization)	1,555	139
Urine (positive ionization)	1,075	120
Plasma (negative ionization)	171	31
Plasma (positive ionization)	412	20

Acknowledgements

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