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**Anna Nudda*[1], S. Carta[1], D. Licastro[2], N. P. Mangia[1], T. Stefaniak[3], J. Bajzert[3], G. Pulina[1]**

[1]*Dipartimento di Agraria, University of Sassari, Italy*

[2]*Area Science Park, Basovizza, 34149 Trieste, Italy,*

[3]*Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, Wroclaw University of Environmental and Life Sciences, Poland*

EAAP
European Federation
of Animal Science

agritech
National Research Center for
Technology in Agriculture

# Could the inclusion of grape pomace in the diet of dairy ewes in late gestation influence the quality of colostrum and its microbiota?

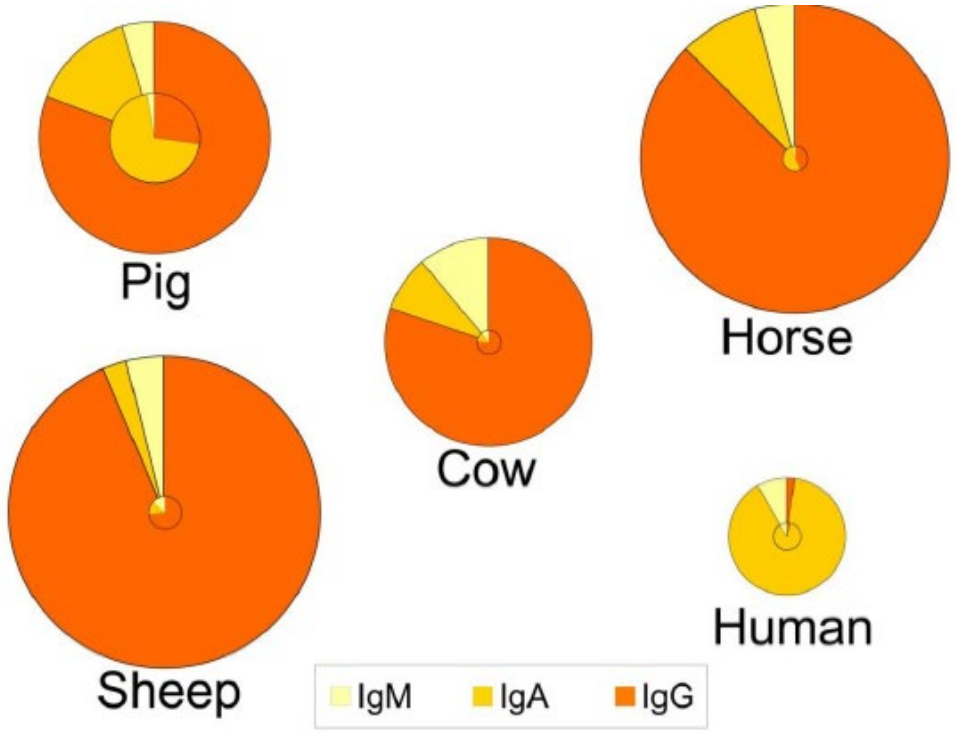
# Colostrum quality

- ✓ **Provide immunogloblulins (IgG, IgM, IgA) that confer passive immunity to newborn lambs**
- ✓ Rich in fats, carbohydrates and proteins, as well as micronutrients such as minerals, it is the fundamental source of nutrients for newborn lambs.
- ✓ A high immune load and good composition of colostrum are essential in dairy sheep flocks to ensure a strong antibody reserve for the newborn in the peripartum period, to guarantee high disease resistance and survival in the replacement animal.

# The sheep colostrum displays the highest IgG content

**Sheep colostrum**

- ✓ **IgG, 90%**
- ✓ IgA, 4%
- ✓ IgM, 6%




Hurley W. L. and Theil P. K. 2011. Perspectives on Immunoglobulins in Colostrum and Milk. Nutrients. 3, 442-474; doi:10.3390/nu3040442

- In Sardinia: about 3 millions of dairy ewes
- represents the 60% of the total dairy sheep bred in Italy
- About 75% of Italian meat from suckling lambs came from Sarda breed
- There is scant data available on the  quality of colostrum in Sarda dairy sheep

# Main factors affecting colostrum quality (IgG content)

- ✓ Species
- ✓ breed
- ✓ Parity
- ✓ Post-partum time sampling
- ✓ Diseases
- ✓ Season
- ✓ **Pre-partum nutrition**

# Pre-partum nutrition

ARTICLE IN PRESS—UNCORRECTED PROOF

J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2023-24349


**INVITED REVIEW: Nutritional and management factors that influence colostrum production and composition in dairy cows**

T. A. Westhoff,[1] S. Borchardt,[2] and S. Mann1*
[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.
[2]Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universitaet Berlin, Koenigsweg 65, 14163 Berlin, Germany

- Dietary energy level
- Dietary **starch content**
- Metabolizable **protein supply**
- Dietary strategies to mitigate **hypocalcemia** (feeding a zeolite or manipulating the dietary calcium or cation anion difference (DCAD))
- **Mineral** and **vitamin** inclusion (vitamin D, vitamins B, Cu, Zn, Mn)
- **Feed additives** (magnesium butyrate, monensin, etc)

# Supplementation of ingredients with antioxidant properties in late gestation improve IgG content in colostrum of sows

- **Grape seed** polyphenols (Wang et al., 2019 – J. Anim. Sci.)
- **Ginger extract** (Dae Lee et al., 2013 - Livestock Science)
- ***Forsythia suspensa*** extract (Long et al., 2020 – ANIFEE)
- **Green alga** (*U. armoricana*) extracts (Bussyet al., 2019 – Vet. Anim. Sci.)
- **Seaweed extract** from a Laminaria spp (Leonard et al., 2010 - J. Anim. Sci.)

# Dietary supplementation of **Moringa oleifera leaf meal** in late gestation improve IgG content in colostrum of **cows**

J. Dairy Sci. 105:5813–5821
https://doi.org/10.3168/jds.2021-21535

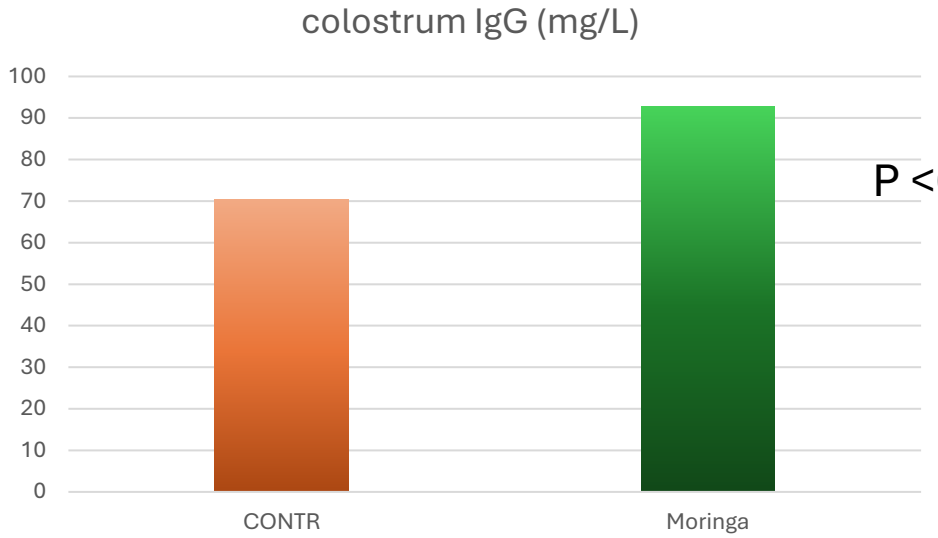

**Prepartum supplementation of *Moringa oleifera* leaf meal: Effects on health of the dam, colostrum quality, and acquisition of immunity in the calf**

T. W. Kekana,[1,2*] U. Marume,[2] and F. V. Nherera-Chokuda[3]
[1]Elsenburg Agricultural Training Institute, Western Cape Department of Agriculture, Private Bag X01, Elsenburg, 7607, South Africa
[2]Department of Animal Science, North-West University, Private Bag X 2046, Mmabatho, 2735, South Africa
[3]National Emergent Red Meat Producers' Organisation, P.O. Box 36461, Pretoria, 0102, South Africa

Table 3. Effect of prepartum *Moringa oleifera* leaf meal (MOLM) supplementation to dairy cows on colostrum IgG

| | Treatment[1] | | | |
|---|---|---|---|---|
| IgG, g/L | CON | MOLM | SEM | *P*-value |
| First harvest | 70.3 | 92.7 | 2.82 | <0.01 |
| Second harvest | 66.3 | 75.2 | 2.14 | 0.04 |
| Third harvest | 33.2 | 42.3 | 1.82 | 0.03 |
| Fourth harvest | 27.8 | 35.4 | 2.22 | 0.04 |
| Fifth harvest | 6.95 | 11.2 | 0.26 | 0.03 |
| Sixth harvest | 8.22 | 13.2 | 0.96 | 0.02 |

[1]CON = control; MOLM = 16.66 g of MOLM/100 kg of BW per day.




P <0.01

*Moringa oleifera* is reach in flavonoids (e.g. myricetin, quercitine…)

# Dietary supplementation of **halzenut skin** in late gestation improve IgG content in colostrum of **ewes**

animals

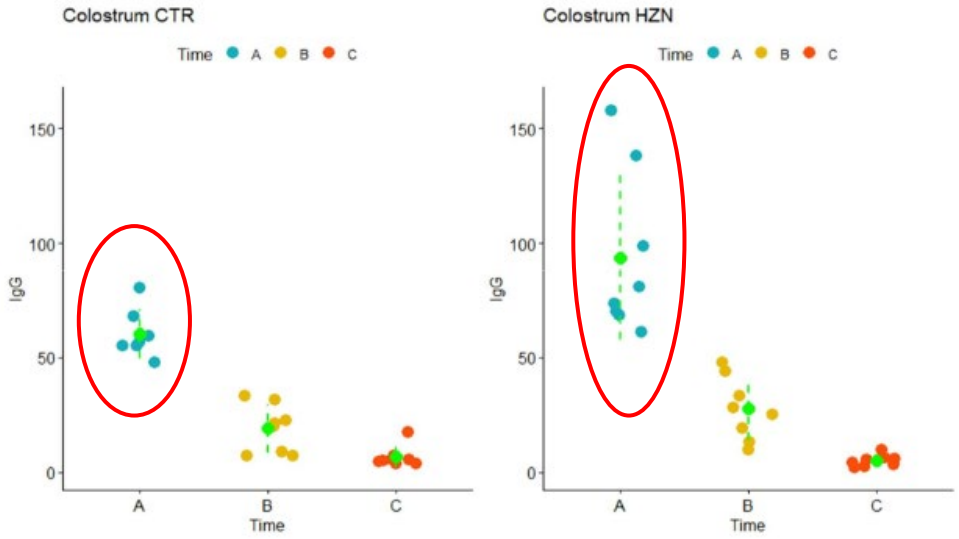
MDPI

Article

**Hazelnut Skin in Ewes' Diet: Effects on Colostrum Immunoglobulin G and Passive Transfer of Immunity to the Lambs**

Irene Viola [1], Paolo Tizzani [1], Giovanni Perona [1], Carola Lussiana [2], Antonio Mimosi [2], Patrizia Ponzio [1] and Paolo Cornale [2,*]

| Sample | Group (n = 8)[1] | A |
|---|---|---|
| Sheep | CTR | 16.5±3.2 |
| | HZN | 16.0±1.6 |
| Colostrum | CTR | 60.4±10.7 |
| | HZN | 93.7±35.9 |
| Lambs | CTR | 5.2±1.5 |
| | HZN | 4.0±1.2 |




**Hazelnut skin** supplementation increase IgG colostrum concentration at lambing, with improved immune passive transfer to the suckling lambs.

# Introduction

| Item[f] | CON | GM |
|---|---|---|
| BCS change | 0.05 | 0.05 |
| BW change, kg | 0.94 | 1.01 |
| DMY, kg/day | 1.88[a] | 1.79[b] |
| Yield, g/day | | |
| Milk | 1220[b] | 1421[a] |
| FPCM | 1245[b] | 1373[a] |
| Fat | 80.92[ab] | 86.36[a] |
| Protein | 71.5[b] | 79.9[a] |
| Casein | 55.35[b] | 61.21[a] |
| Lactose | 57.84[b] | 66.63[a] |
| Urea | 0.61[ab] | 0.665[a] |
| Milk composition | | |
| Fat, g/kg | 67.9[a] | 59.2[c] |
| Protein, g/kg | 60.4[a] | 56.3[b] |
| Casein, g/kg | 47.1[a] | 43.5[b] |
| Lactose, g/kg | 47.8[a] | 48.2[a] |
| Urea, mg/dL | 48.4[ab] | 46.7[b] |
| Log SCC, × 1000 cell/mL | 2.01 | 2.11 |
| Log TBC, x 1000 UFC/mL | 2.80[a] | 2.43[b] |

Animal Feed Science and Technology 251 (2019) 76–85

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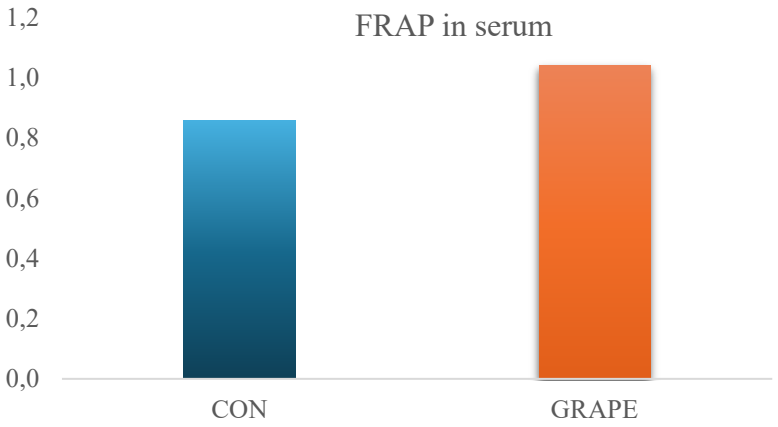
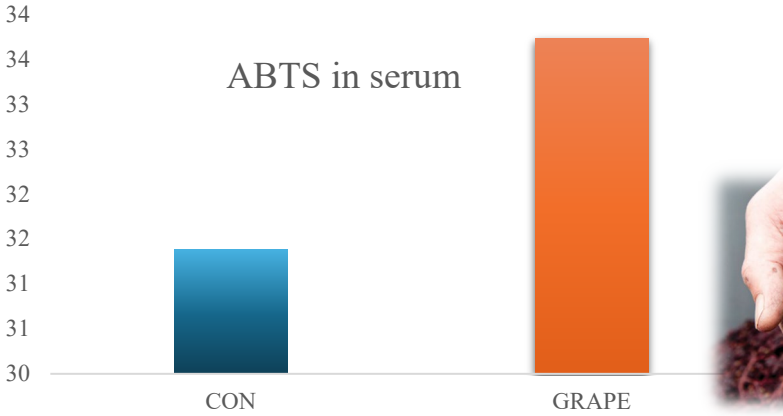
Small amounts of agro-industrial byproducts in dairy ewes diets affects milk production traits and hematological parameters

A. Nudda[a,*], G. Buffa[a], A.S. Atzori[a], M.G. Cappai[b], P. Caboni[c], G. Fais[c], G. Pulina[a]

The grape marc supplementation (100 g/d) to lactating Sarda ewes increased milk production (+200 g/day), as well as protein (+8.4 g/day) and fat (+5.5 g/day) milk contents compared to the CON group.

# Introduction




Received: 3 October 2019 | Revised: 18 December 2019 | Accepted: 31 December 2019
DOI: 10.1111/jpn.13315

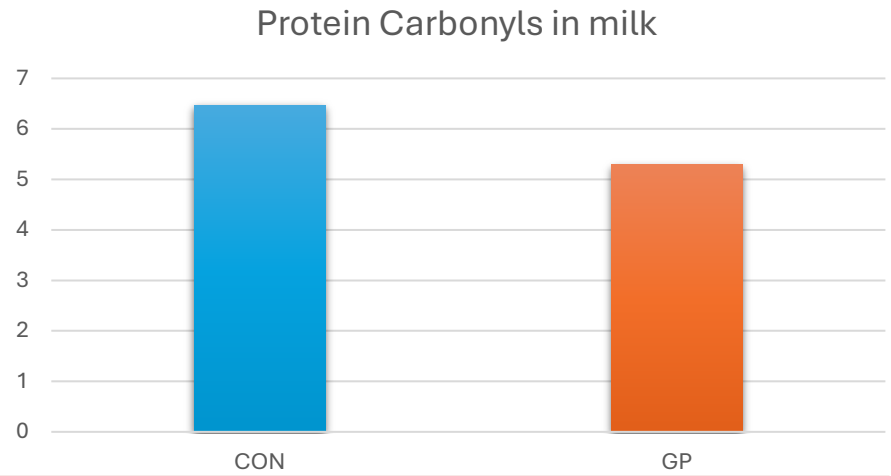
ORIGINAL ARTICLE

Journal of Animal Physiology and Animal Nutrition WILEY

## Supplementation of by-products from grape, tomato and myrtle affects antioxidant status of dairy ewes and milk fatty acid profile

Giovanna Buffa[1] | Eleni Tsiplakou[2] | Christina Mitsiopoulou[2] | Giuseppe Pulina[1] | Anna Nudda[1]

The grape marc supplementation (100 g/d) to lactating Sarda ewes improve the antioxidant status of blood (FRAP and ABTS) and milk (less PC) compared to the CON group.



# Introduction

## Nutritional factors affects milk microbiota

- ✓ Diet could affect the gastrointestinal microbiome and, consequently, nutritional factors may affect milk microbiome (Trevisi et al., 2021)
- ✓ There is not literature about the effect of polyphenols on colostrum and milk microbiota

Phylogenetic variation in raw cow milk microbiota and the impact of forage combinations and use of silage inoculants

Alexandre J. K. Ouamba[1,2], Mérilie Gagnon[1,2], Thibault Varin[1], P. Yvan Chouinard[2,3], Gisèle LaPointe[2,4] and Denis Roy[1,2]*

[1]Département des Sciences des Aliments, Laboratoire de Génomique Microbienne, Université Laval, Québec, QC, Canada, [2]Regroupement de Recherche pour Un Lait de Qualité Optimale (Op+Lait), Saint-Hyacinthe, QC, Canada, [3]Département des Sciences Animales, Université Laval, Québec, QC, Canada, [4]Department of Food Science, University of Guelph, Guelph, ON, Canada

Shift in the cow milk microbiota during alpine pasture as analyzed by culture dependent and high-throughput sequencing techniques

Ilaria Carafa[a], Irma Castro Navarro[a], Giovanni Bittante[b], Franco Tagliapietra[b], Luigi Gallo[b], Kieran Tuohy[a], Elena Franciosi[a,*]

[a] *Research and Innovation Centre, Food Quality and Nutrition Department, Fondazione Edmund Mach (FEM), San Michele all' Adige, TN, Italy*
[b] *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, PD, Italy*

J. Dairy Sci. 103:5931–5946
https://doi.org/10.3168/jds.2019-17918


**Prevalence and abundance of lactic acid bacteria in raw milk associated with forage types in dairy cow feeding**

**Mérilie Gagnon,[1,2] Alexandre J. K. Ouamba,[1,2] Gisèle LaPointe,[2,3] P. Yvan Chouinard,[2,4] and Denis Roy[1,2]***
[1]Département des Sciences des Aliments, Laboratoire de Génomique Microbienne, Université Laval, Québec, QC G1V 0A6, Canada
[2]Regroupement de Recherche pour un Lait de Qualité Optimale (Op+Lait), Saint-Hyacinthe, QC J2S 2M2, Canada
[3]Department of Food Science, University of Guelph, Guelph, ON N1G 2W1, Canada
[4]Département des Sciences Animales, Université Laval, Québec, QC G1V 0A6, Canada

RESEARCH ARTICLE

**Effect of Pasture Versus Indoor Feeding on Milk Microbiota of Goats**

Tian JING [1,a,#] Xiaoting YAO [1,b,#] Tiantian JI [1,c] Jiarui DONG [1,d] Xi CHEN [1,e] Fengqiang CHEN [1,f] Hao FENG [1,g] Tianxing WANG [1,h] Huiying ZHAO [1,i (*)] Dekun CHEN [1,j (*)] Wentao MA [1,k (*)]

*[#] These authors contributed equally to this work*
[1] Veterinary Immunology Laboratory, College of Veterinary Medicine, Northwest Agriculture and Forestry University, Yangling 712100, Shaanxi Province, CHINA
ORCIDs: [a] 0000-0002-5634-9725; [b] 0000-0002-2853-6240; [c] 0000-0002-8634-9558; [d] 0000-0002-8162-6157; [e] 0000-0002-7207-4709 [f] 0000-0001-6342-583X; [g] 0000-0001-5315-540X; [h] 0000-0001-8636-6801; [i] 0000-0003-2449-4108; [j] 0000-0002-3215-9337; [k] 0000-0002-4747-1489

# Polyphenols from grape could affect the ruminal microbial populations

PLOS ONE

RESEARCH ARTICLE

Rumen microbiome in dairy calves fed copper and grape-pomace dietary supplementations: Composition and predicted functional profile

Filippo Biscarini[1,2☯], Fiorentina Palazzo[3☯], Federica Castellani[3], Giulia Masetti[2,4], Lisa Grotta[3], Angelo Cichelli[5], Giuseppe Martino[3*]

J. Dairy Sci. 102:3781–3804
https://doi.org/10.3168/jds.2018-14985


*Invited review:* Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: Experimental evidence and methodological approaches

V. Vasta,[1] M. Daghio,[2] A. Cappucci,[3] A. Buccioni,[2] A. Serra,[3] C. Viti,[2] and M. Mele[3,4*]
[1]Food Scientist, viale delle Alpi 40, 90144, Palermo, Italy
[2]Dipartimento di Scienze delle Produzioni Agro-Alimentari e dell'Ambiente, University of Florence, Piazzale delle Cascine 18, 50144 Firenze, Italy
[3]Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
[4]Centro di Ricerche Agro-ambientali "E. Avanzi," University of Pisa, Via Vecchia di Masrina, 6, 56100 Pisa, Italy

ITALIAN JOURNAL OF ANIMAL SCIENCE
2020, VOL. 19, NO. 1, 1462–1471
https://doi.org/10.1080/1828051X.2020.1848465

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Agroindustrial by-products from tomato, grape and myrtle given at low dosage to lactating dairy ewes: effects on rumen parameters and microbiota

G. Buffa[a], N. P. Mangia[a], A. Cesarani[a], D. Licastro[b], S. Sorbolini[a], G. Pulina[a] and A. Nudda[a]

Khiaosa-ard et al.
Journal of Animal Science and Biotechnology (2023) 14:92
https://doi.org/10.1186/s40104-023-00892-7

Journal of Animal Science and Biotechnology

RESEARCH Open Access

Winery by-products as a feed source with functional properties: dose–response effect of grape pomace, grape seed meal, and grape seed extract on rumen microbial community and their fermentation activity in RUSITEC

Ratchaneewan Khiaosa-ard[1*], Mubarik Mahmood[1,2], Elsayed Mickdam[1,3], Cátia Pacífico[1,4], Julia Meixner[1] and Laura-Sophie Traintinger[1]

*diversity*

MDPI

*Article*

Effect of Grape Pomace Intake on the Rumen Bacterial Community of Sheep

Michal Rolinec [1,*], Juraj Medo [2], Michal Gábor [1], Martina Miluchová [1], Milan Šimko [1], Branislav Gálik [1], Ondrej Hanušovský [1], Zuzana Schubertová [3], Daniel Biro [1], Luboš Zábranský [4] and Miroslav Juráček [1,*]

# Objectives

The objectives of this study were:

- to evaluate the effect of the inclusion of GP in the diet of sheep in the late gestation on **composition and immunological quality of colostrum**

- To investigate if GP affects colostrum microbiome

# Materials and methods

- The study was conducted at the experimenral farm of the Dipartimento di Agraria, University of Sassari

- Ewes were kept together in a barn equipped with 10 individual automatic feeding systems (Biocontrol AS, Rakkestad, Norway), and the **individual feed intake** was daily recorded

# Materials and methods

- **21 Sarda dairy sheep** in late gestation (13-21 days from partum) were used for the experiment
- The animals were allocated to two groups homogeneous for:

❖ **Body condition score** (BCS, 3.4 ± 0.1)

❖ **Body weight** (BW, 62.2 ± 0.7 kg, mean ± SD)

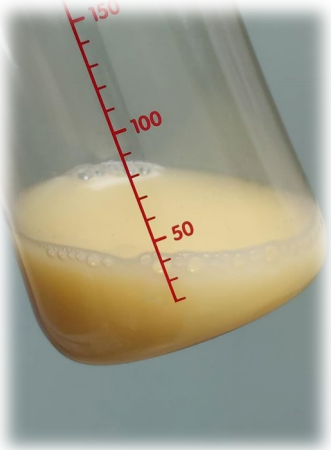
## CON group

- 11 ewes
- Fed basal diet
- 0 g/d of GP

## Grape group

- 10 ewes
- Fed basal diet
- **50 g/d of GP**

# Materials and methods




## Samples collection

**Colostrum** and **Blood**

ewes lambs

- ✓ Collected from each ewe within 12 h after lambing

Analyses:

- IgG (by ELISA test)
- Protein, Fat , lactose, SCC, urea, NaCl, casein, (Milkoscan FT 6000 – Laore Regional Laboratory)
- Fatty acids profile (GC Agilent – 100 m column )
- DNA extraction (QIAamp Fast DNA Stool Mini Kit)
- Microbiome analysis NGS (Illumina 16S Metagenomic Sequencing)

- ✓ Ewes within 12 h after lambing
- ✓ Lambs after 24 h from parturition

- ✓ Analyses:
- IgG by ELISA test

# Statistical Analysis

- ✓ Differences in the concentrations of the **colostrum components ad IgG** between groups were analyzed with one-way ANOVA and compared by Tukey test
- ✓ Correlation analysis was performed between IgG content for colostrum and serum samples
- ✓ Microbiome analysis (alpha and beta diversity with Bioconductor package phyloseq and DESeq2 package for microbial group differences)

# Results

## Colostrum composition of control and GP supplemented groups

| | CON | GRAPE | P-value |
|---|---|---|---|
| **Fat, %** | 10.41 | 10.69 | 0.85 |
| **Protein, %** | 13.51 | 15.60 | 0.38 |
| **Lactose, %** | 3.34 | 2.76 | 0.14 |
| **Log SCC,** | 2.51 | 2.38 | 0.68 |
| **Urea** | 58.0 | 71.1 | 0.26 |
| **pH** | 5.83 | 5.66 | 0.09 |
| **NaCl** | 611 | 615 | 0. 95 |

# Results

## Fatty acids profile in colostrum

| FAME, (g/100 g of FA) | CON | GP | P-Value |
|---|---|---|---|
| **C4:0** | 2.06 | 1.9 | 0.760 |
| **C10:0** | 2.37 | 1.86 | 0.170 |
| **C12:0** | 2.49 | 2.23 | 0.200 |
| **isoC13:0** | 0.011 | 0.011 | 0.540 |
| **anteisoC13:0** | 0.036 | 0.035 | 0.422 |
| **C13:0** | 0.052 | 0.040 | 0.159 |
| **isoC14:0** | 0.058 | 0.061 | 0.995 |
| **C14:0** | 13.6 | 12.8 | 0.416 |
| **isoC15:0** | 0.127 | 0.112 | 0.258 |
| **anteisoC15:0** | 0.181 | 0.160 | 0.243 |
| **C15:0** | 0.688 | 0.570 | 0.123 |
| **isoC16:0** | 0.184 | 0.186 | 0.951 |
| **C16:0** | 33.1 | 32.4 | 0.686 |
| **isoC17:0** | 0.378 | 0.361 | 0.681 |
| **anteisoC17:0** | 0.501 | 0.491 | 0.834 |
| **C16:1c9** | 1.95 | 1.98 | 0.453 |
| **C17:0** | 0.79 | 0.84 | 0.455 |
| **isoC18:0** | 0.016 | 0.021 | 0.012 |
| **C18:0** | 6.31 | 6.48 | 0.632 |
| **C18:1t11** | 0.36 | 0.35 | 0.821 |
| **C18:1c9** | 25.0 | 27.2 | 0.425 |
| **C18:2n6** | 2.07 | 2.22 | 0.315 |
| **C18:3n6** | 0.17 | 0.165 | 0.825 |
| **C18:3n3** | 0.38 | 0.42 | 0.278 |
| **CLAc9t11** | 0.376 | 0.361 | 0.997 |
| **CLAt9c11** | 0.009 | 0.020 | 0.005 |
| **CLAt11t13** | 0.012 | 0.018 | 0.055 |
| **EPA** | **0.046** | **0.058** | **0.033** |
| **C24:0** | 0.012 | 0.009 | 0.490 |
| **DPA** | **0.140** | **0.176** | **0.095** |
| **DHA** | 0.053 | 0.054 | 0.600 |

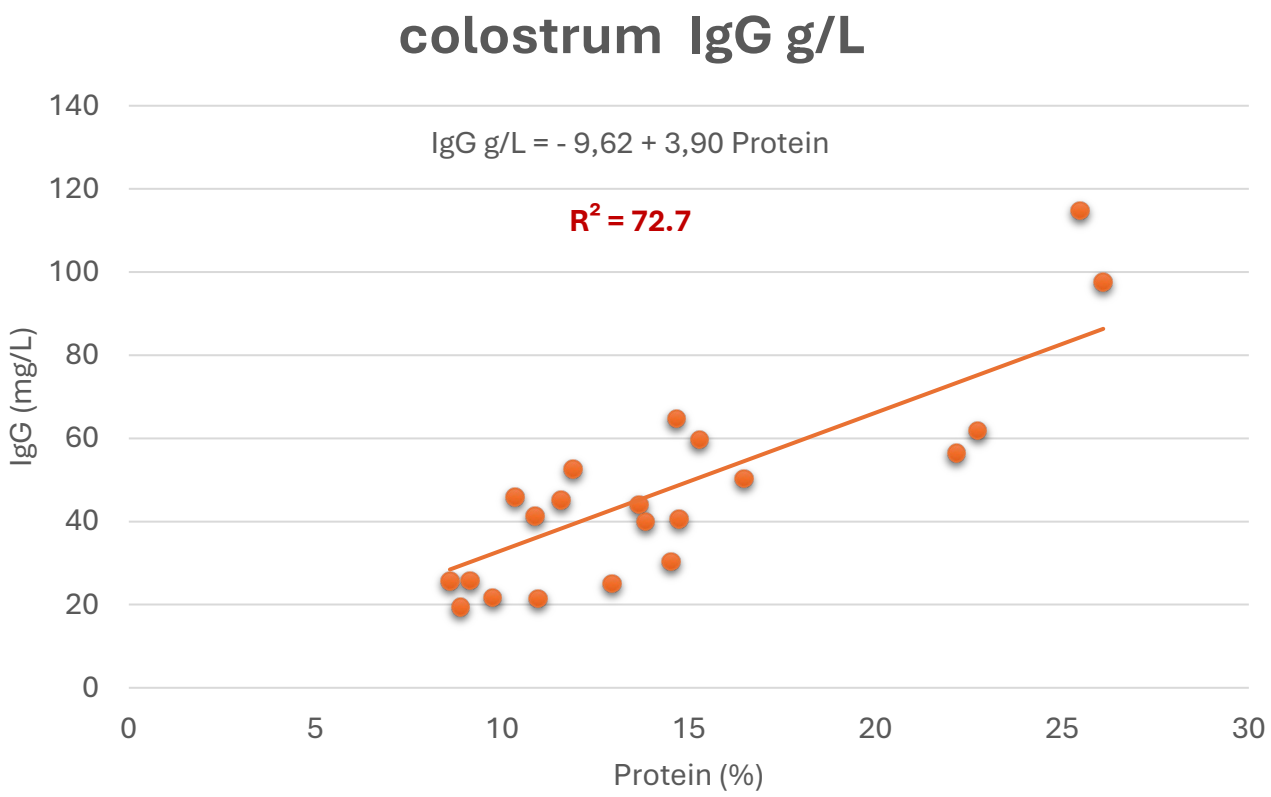
# Results

## IgG in colostrum and serum of ewes and lamb of control and GP supplemented groups

| | CON | GRAPE | P-value |
|---|---|---|---|
| **Colostrum IgG, g/L** | 36.5 | 58.3 | 0.04 |
| **Serum of ewes IgG, g/L** | 13.61 | 15.05 | 0.53 |
| **Serum of lamb IgG, g/L** | 22.4 | 32.8 | 0.05 |

# Results

## Relationship between Total protein and IgG in colostrum

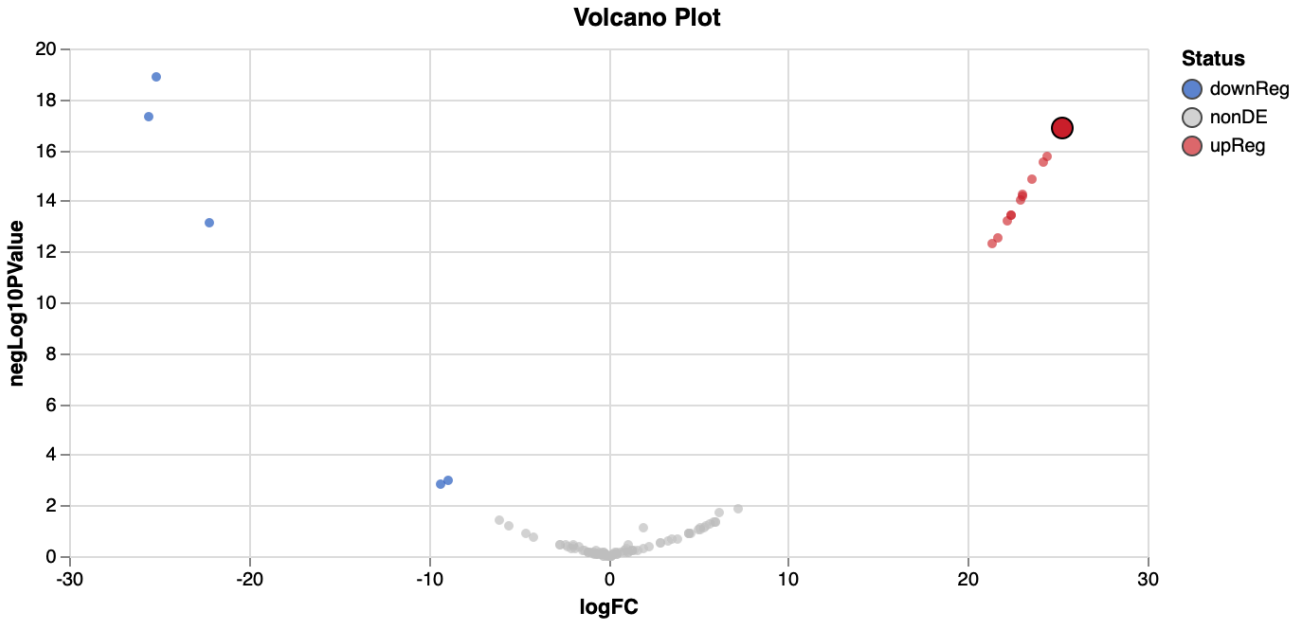


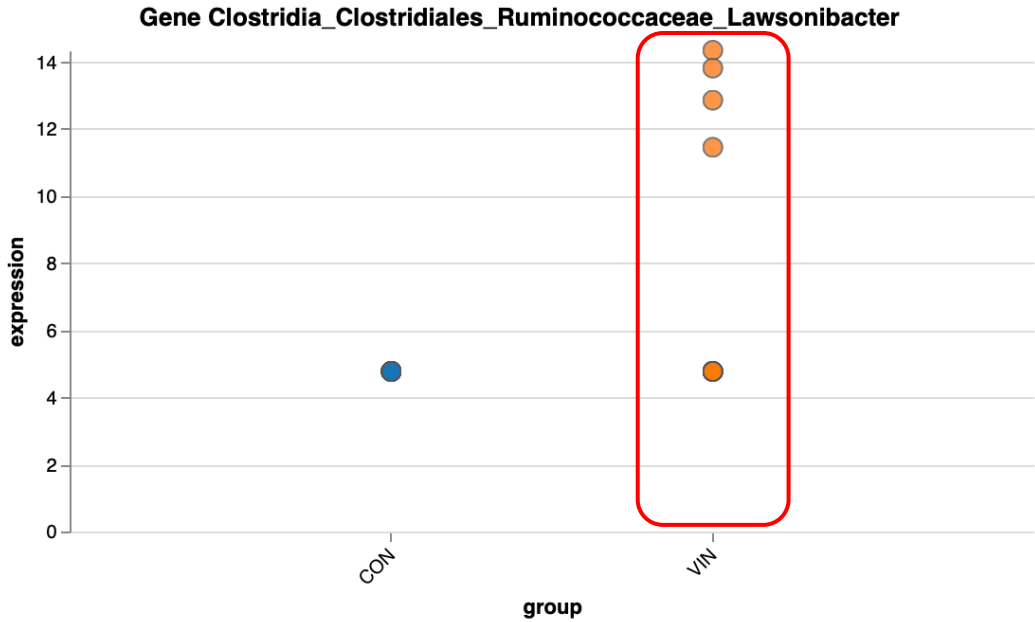

A close correlation was found between Protein and IgG concentration in colostrum.

# Colostrum microbiota analysis: Taxonomic classification

- ✓ The colostrum samples from **GP** had higher abundance of genera ***Lawsonibacter***, ***Harryflintia***, ***Anaerotruncus*** and ***Monoglobus***, belonging to ***Ruminococcaceae*** family, compared to samples from CON

## Clostridia Clostridiales Ruminococcaceae Lawsonibacter



# Colostrum microbiota analysis: Taxonomic classification

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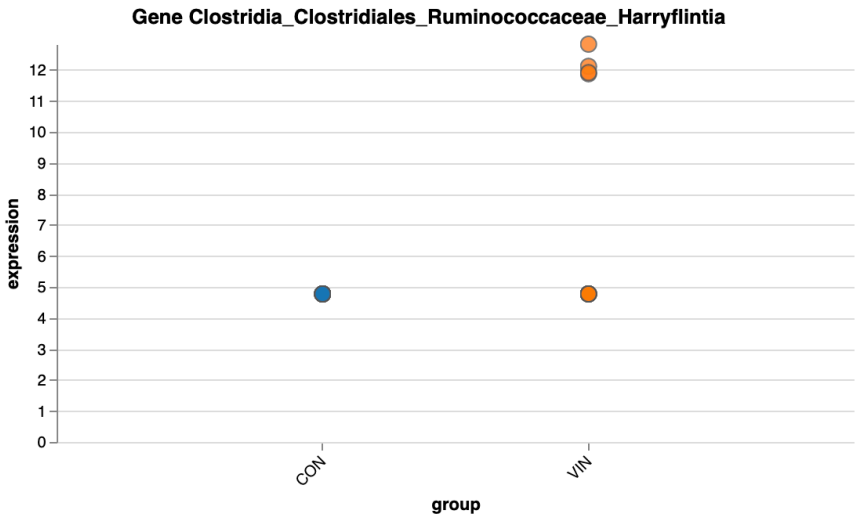
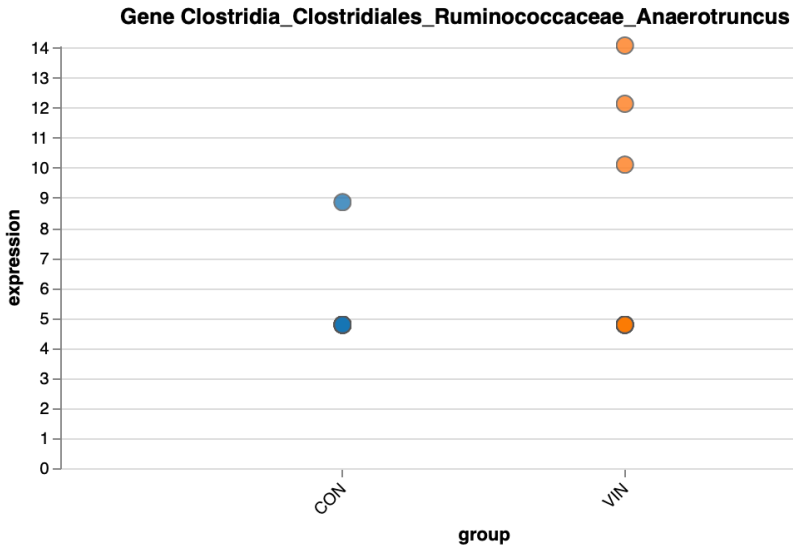
**Clostridia Clostridiales Ruminococcaceae family**

**Harryflintia**



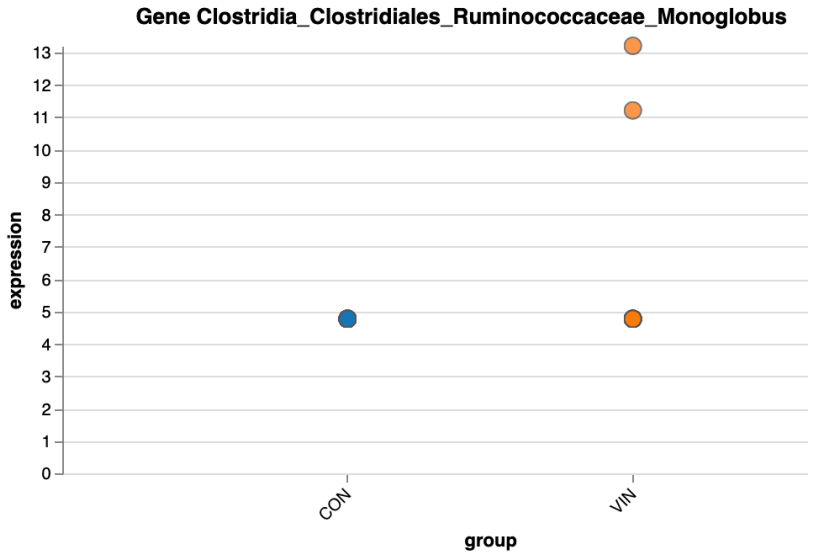

**Clostridia Clostridiales Ruminococcaceae family**

**Anaerotruncus**




**Clostridia Clostridiales Ruminococcaceae family**

**Monoglobus**

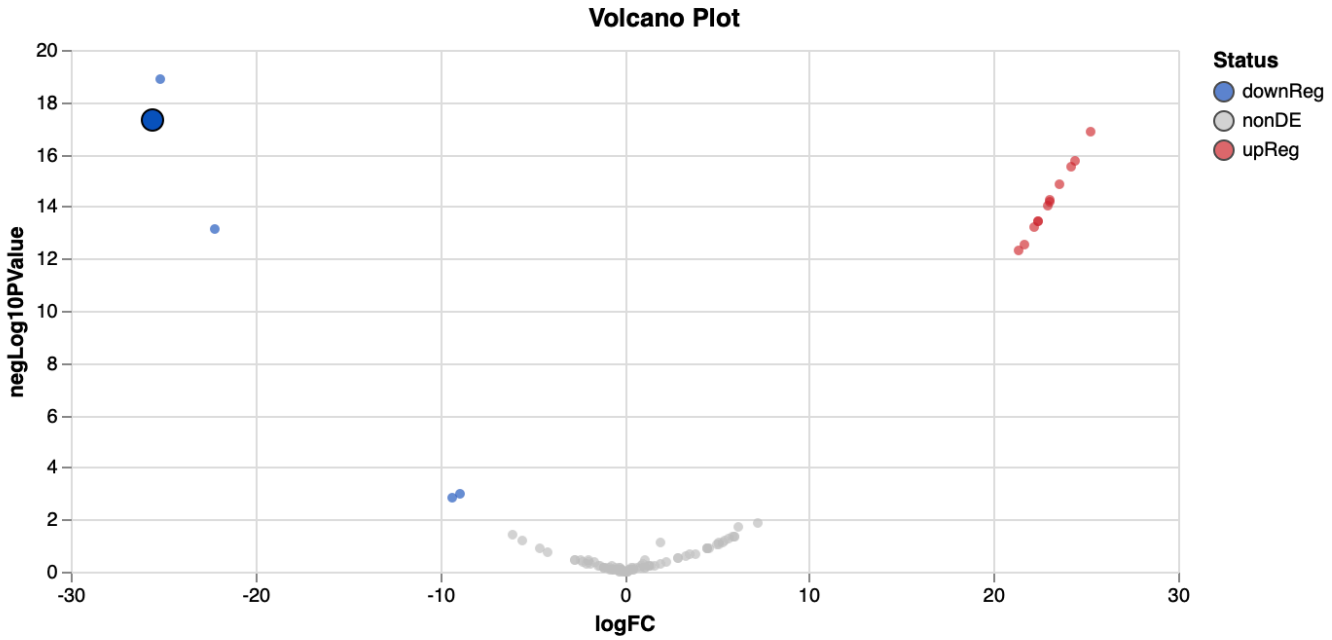


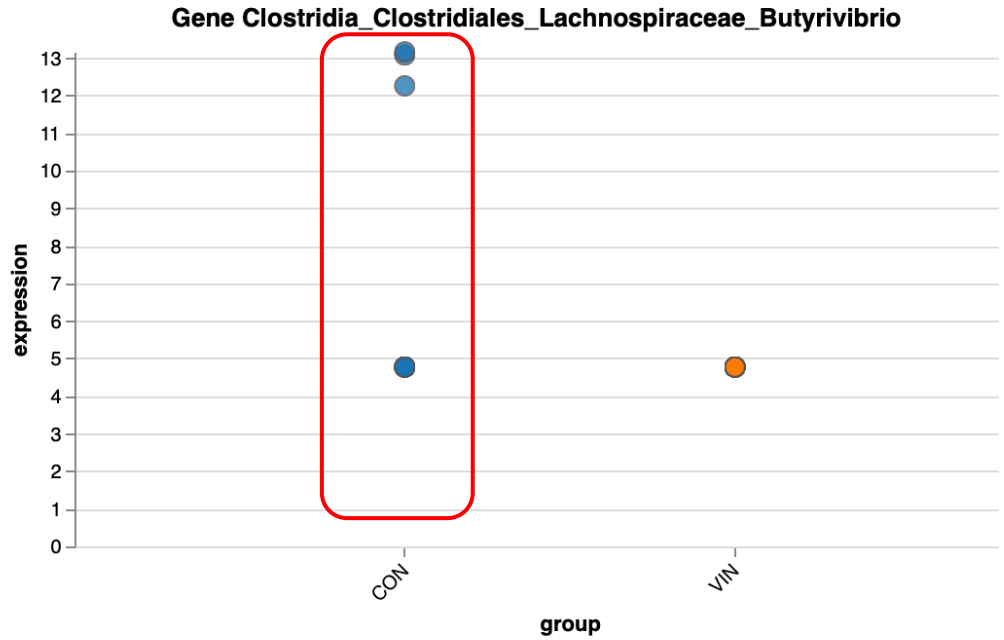
# Colostrum microbiota analysis: Taxonomic classification

✓ The genera ***Butyrivibrio*** and ***Anaerotignum***, belonging to *Lachnospiraceae* family, were higher in **CON** compared to GP

**Clostridia Clostridiales**
**Lachnospiraceae**

**Butyrivibrio**



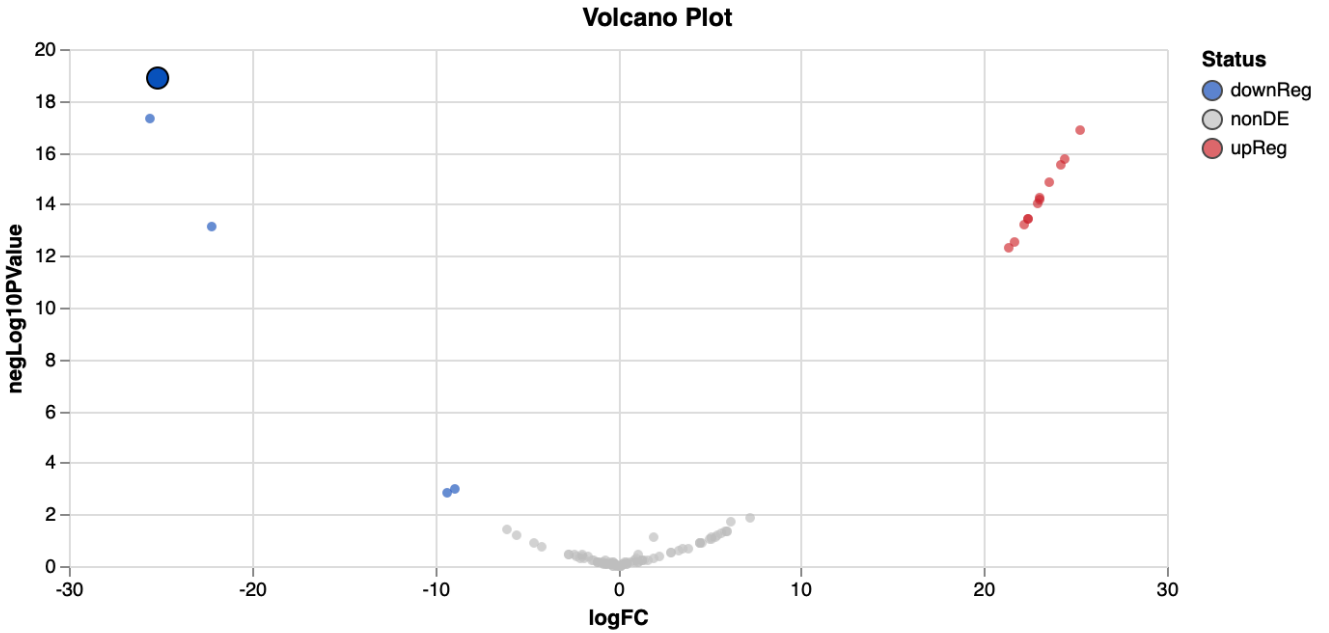



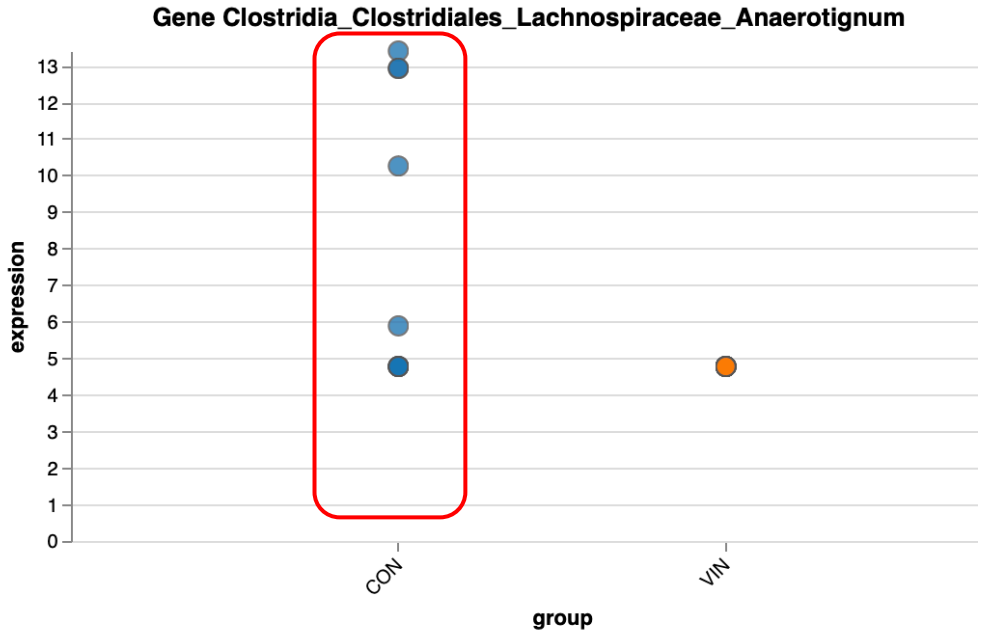
# Colostrum microbiota analysis: Taxonomic classification

✓ The genera ***Butyrivibrio*** and ***Anaerotignum***, belonging to *Lachnospiraceae* family, were higher in **CON** compared to GP

## Clostridia Clostridiales Lachnospiraceae

## Anaerotignum



# Conclusions

- ✓ The inclusion of a 50 g/d of GP in late gestation ewes improve IgG content in colostrum and in serum of lambs
- ✓ The gross composition and the FA profile of colostrum was not influence by GP supplementation
- ✓ The microbial analysis of colostrum evidenced variations in the structure of colostrum microbiota of GP group in comparison to CON diet
- ✓ The effect of antioxidant compound during gestation on colostrum quality deserves more studies (e.g. source, dose, and duration of supplementation)
- ✓ The results on IgG observed should be supported by knowledge on mechanism of action of antioxidant compounds included in the diet of late gestation animals

# Thanks for your attention!

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Anna Nudda: anudda@uniss.it