UNIVERSITÀ DEGLI STUDI DI MILANO

Marie Skłodowska-Curie Actions

75th EAAP, Florence, Italy

Evaluating the effects of rumen originated lipopolysaccharide on the pathogenesis of rumen acidosis

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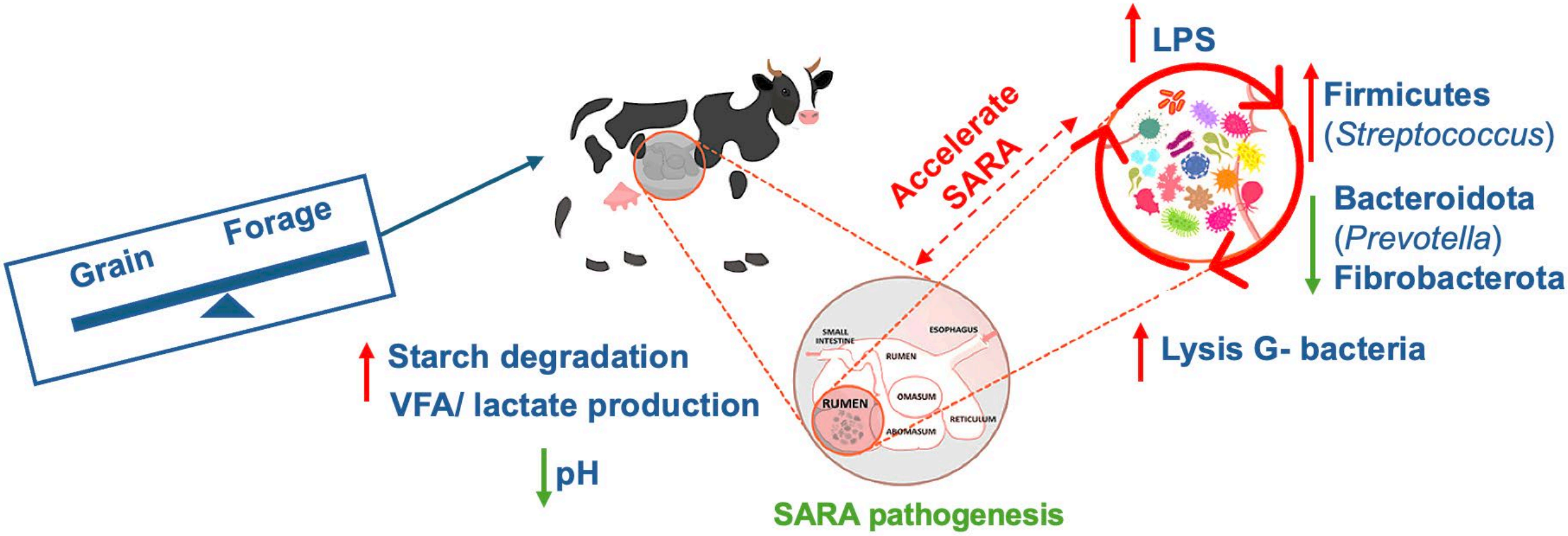
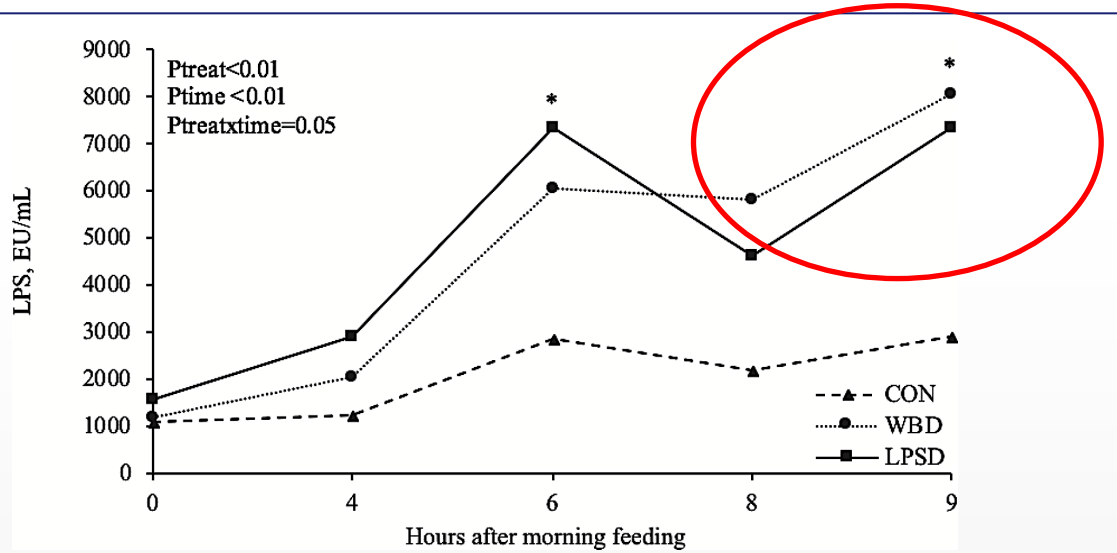
Development of Subacute ruminal acidosis



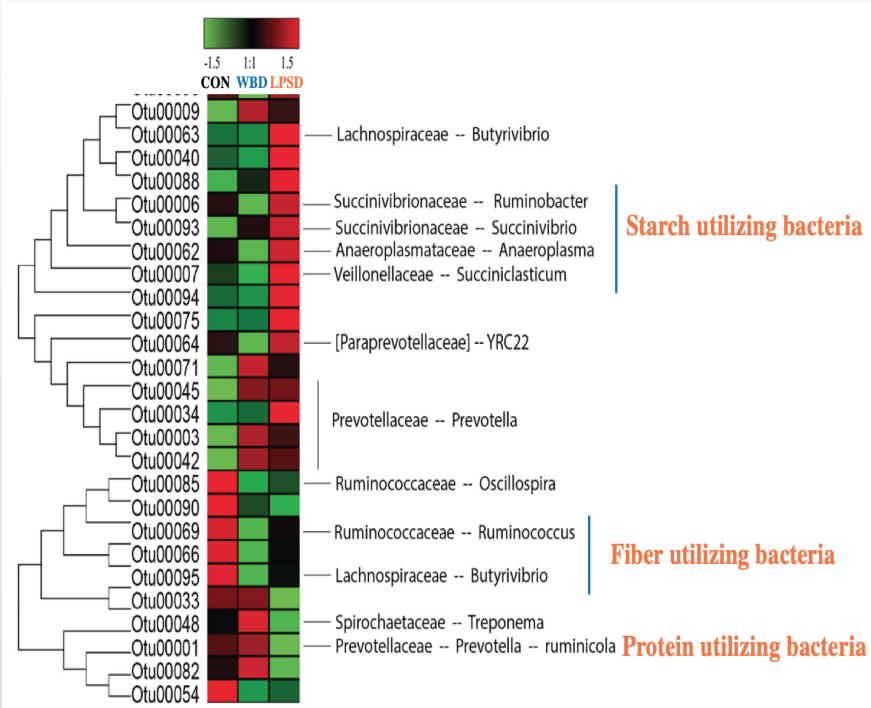
Role of LPS on the SARA pathogenesis

LPS Concentration , EU/mL		Reference
Control	SARA	
24,547	128,825	Gozho et al., 2007
28,184	107,152	Khafipour et al., 2008
42,122	145,593	Khafipour et al., 2009
10,405	168,391	Khafipour et al., 2011



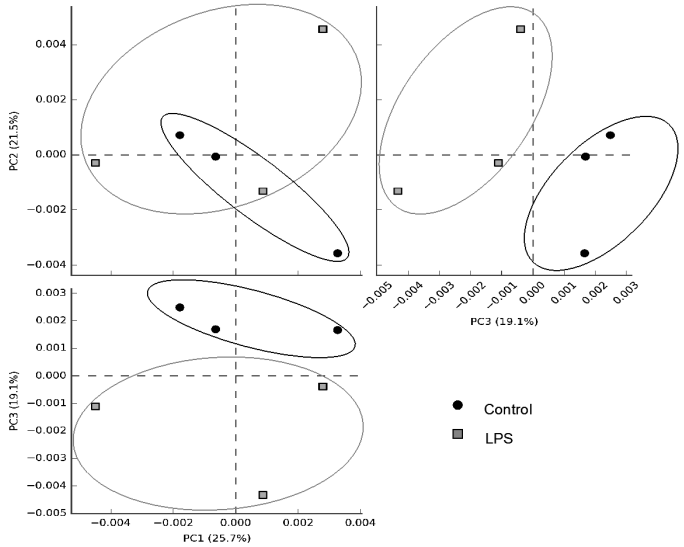
- **Lipopolysaccharide** ⟹ **pathogenesis of ruminal acidosis**

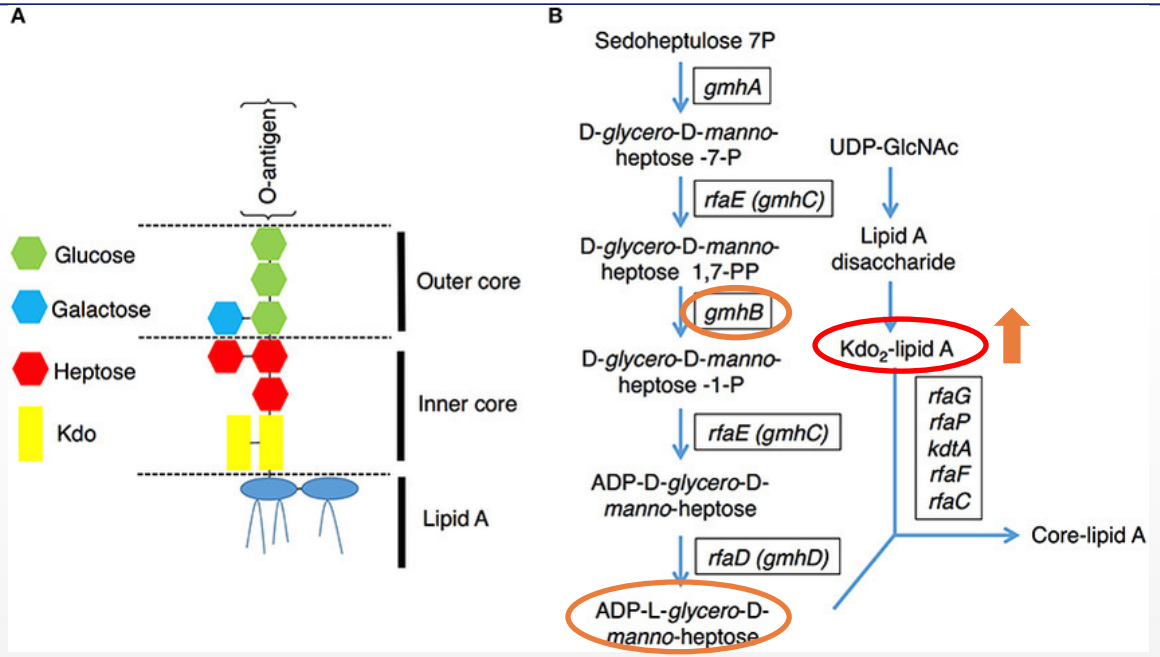
(Owen et al., 1998; Dunlop, 1972)



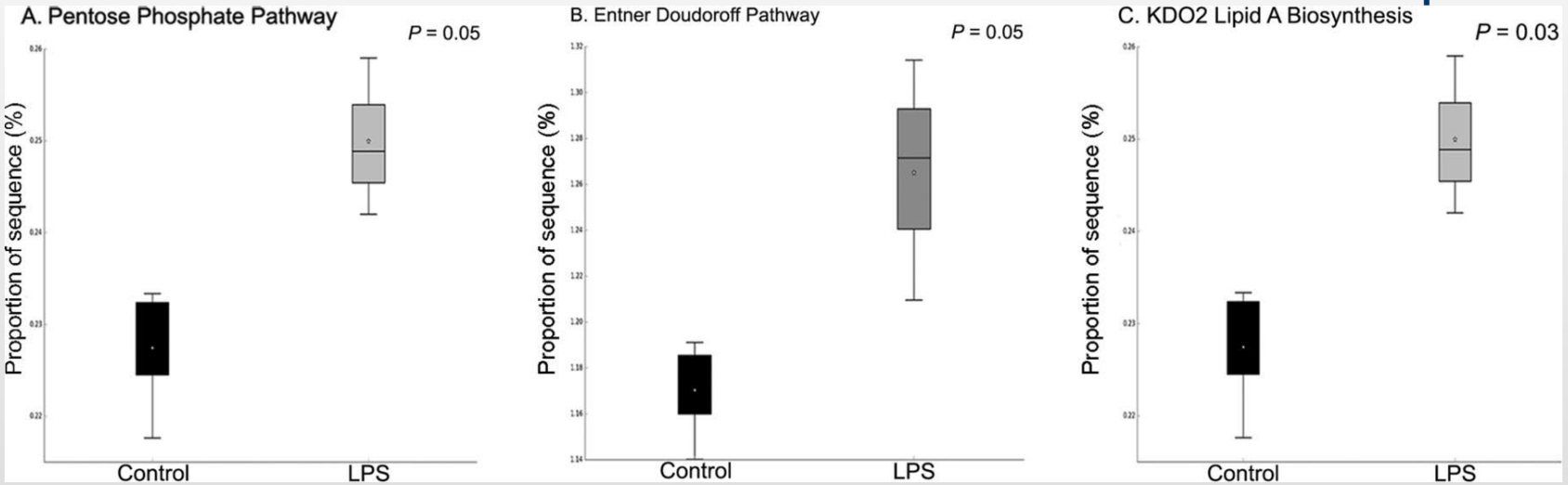
Dai et al., 2019

Role of LPS on the SARA pathogenesis





Core-lipid A biosynthesis pathway



Microbial death/growth triggers a cycle that increases LPS release from surviving microbes.

Dai et al., 2020

Objectives

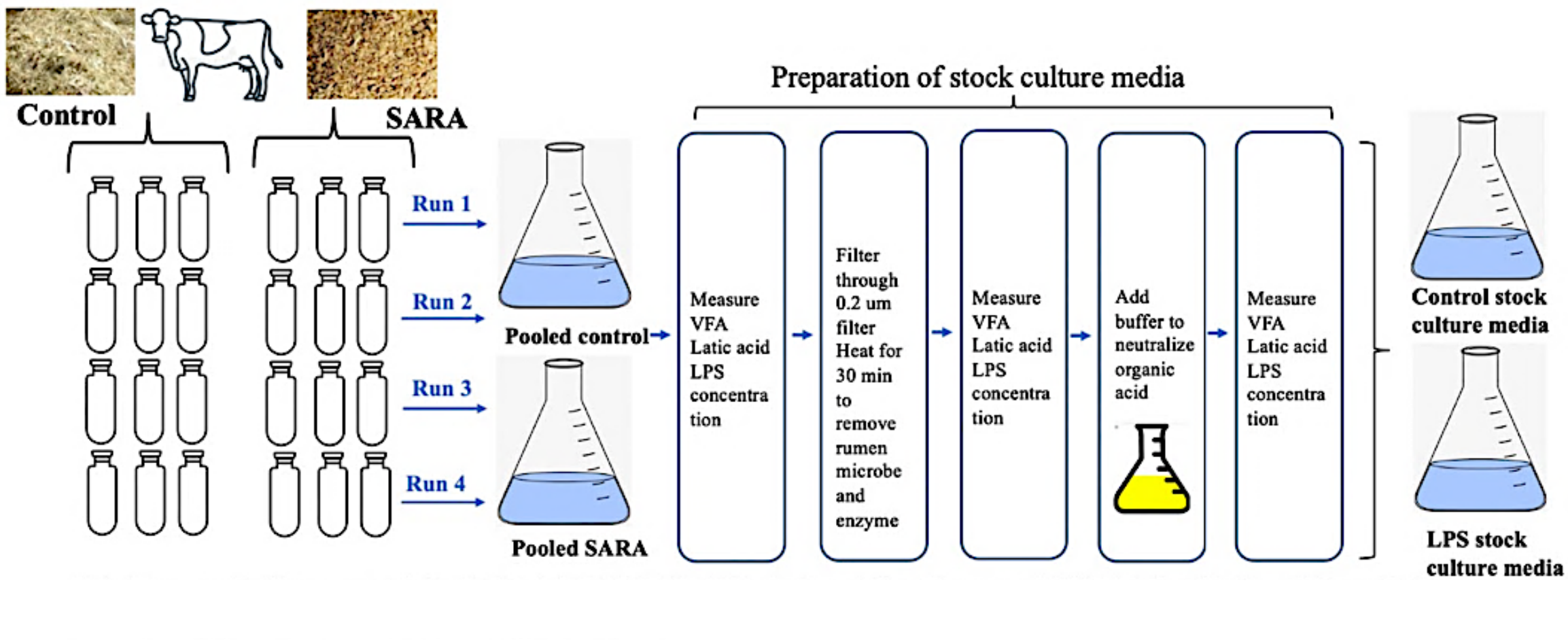
Potency of LPS originated from the ruminal bacteria is different from that of *E. coli* (Nagaraja and Titgemeyer,2007)

LPS released from grain-induced SARA had different potency from alfalfa-pellet-induced SARA due to different ruminal BCC under different substrates (Khafipour et al., 2009 a.b)

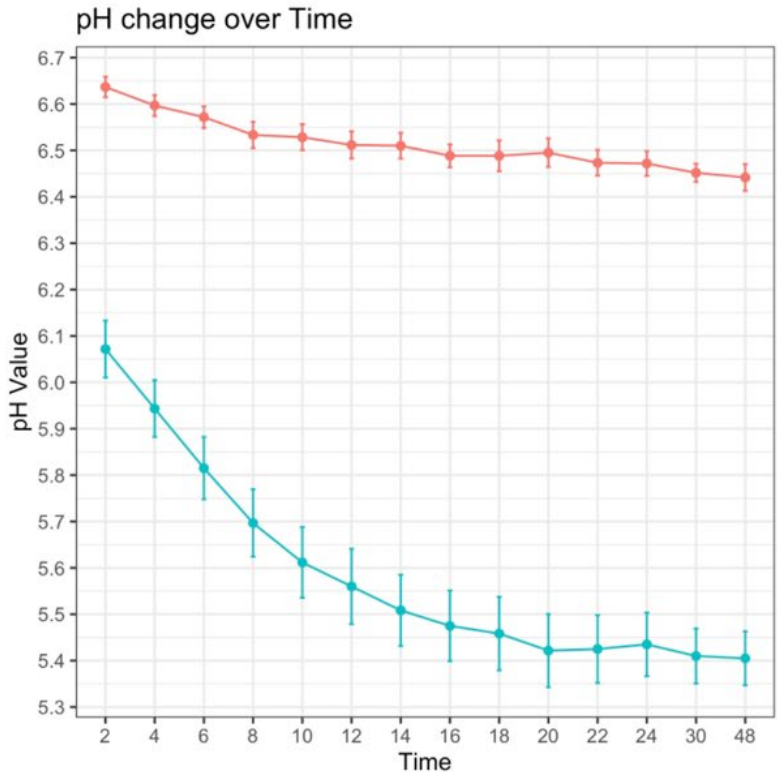
Objectives:

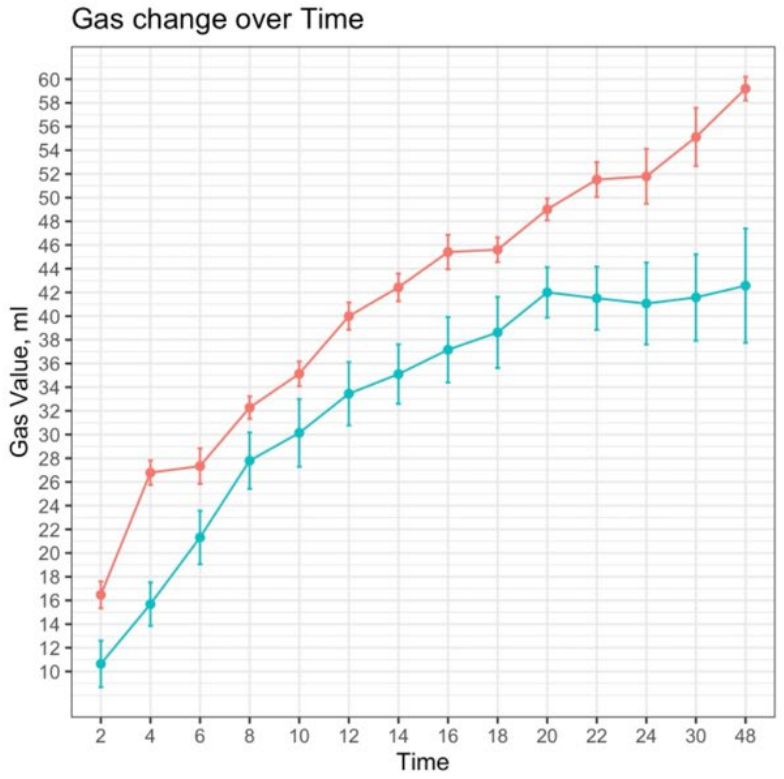
1) Collect LPS from the rumen bacteria by inducing the SARA nutritional model in the batch culture system
2) Compare the effects of different LPS sources on ruminal fermentation, bacterial community composition and function by using 16S rRNA and RNA sequencing to
    - 1) elucidate the roles of ruminal LPS in the pathogenesis of SARA;
    - 2) evaluate whether LPS-E is a representative source to study the role of LPS in the pathogenesis of SARA.

Collected LPS-enriched culture media



Succussed in inducing SARA by using batch culture

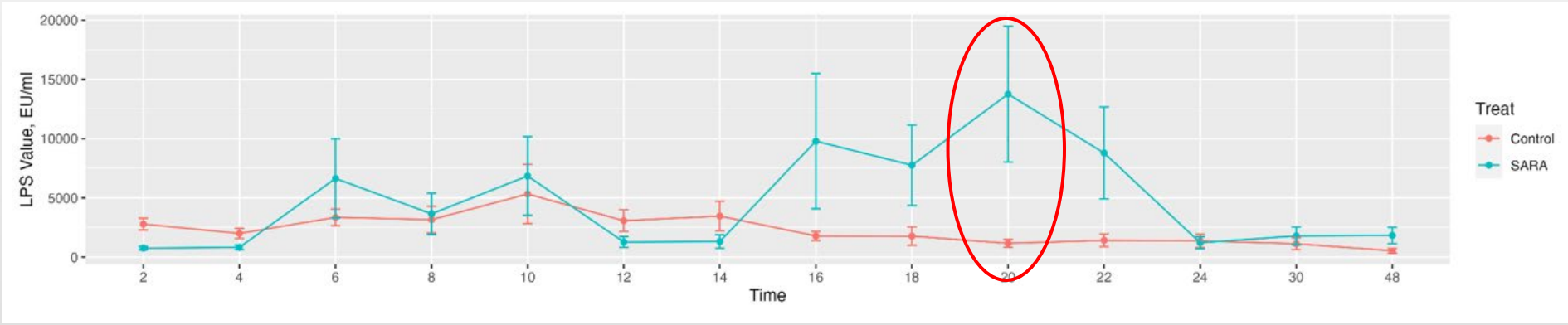




The same ingredient as Control, Added wheat starch in SARA

	Control	SARA
NDF	40.7	29.7
PeNDF	38.0	27.7
NFC	37.0	71.8
Starch	18.1	40.1

1/12 Menke & Steingass Buffer



20 h after incubation

Media preparation (15,086 EU/ml of LPS)

1000 x g at 4 C for 10 min

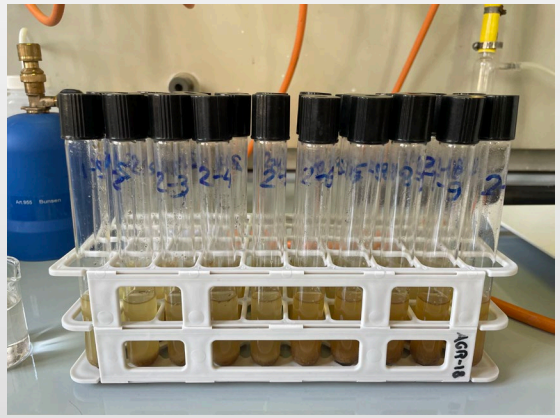
11,250 x g at 4 C for 20 min

0.22 um PES filter

Balance LPS, VFA, lactic acid and pH (6.8)

Add substrate, minerals and vitamin

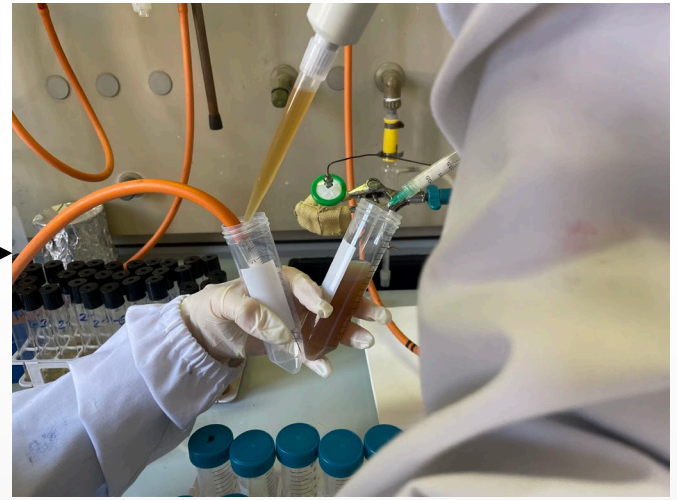
Gassing, tubing and autoclave



Rumen mixed bacteria isolation

Gassing O2-free CO2 for 30 min

Rumen mixed bacteria in the central

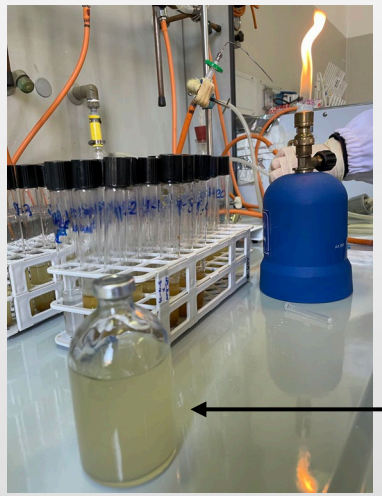


750 x g at 25 C for 10 min → Remove protozoa

10,000 x g at 25 C for 10 min → Harvest rumen mixed bacteria

Wash twice with simplex N-free buffer

Resuspend simplex N-free buffer



Treatments and Sampling

Treatments:

LPS.F: LPS free

LPS.E: 14,826 ± 964 EU/ml

LPS.R: 15,086 ± 964 EU/ml

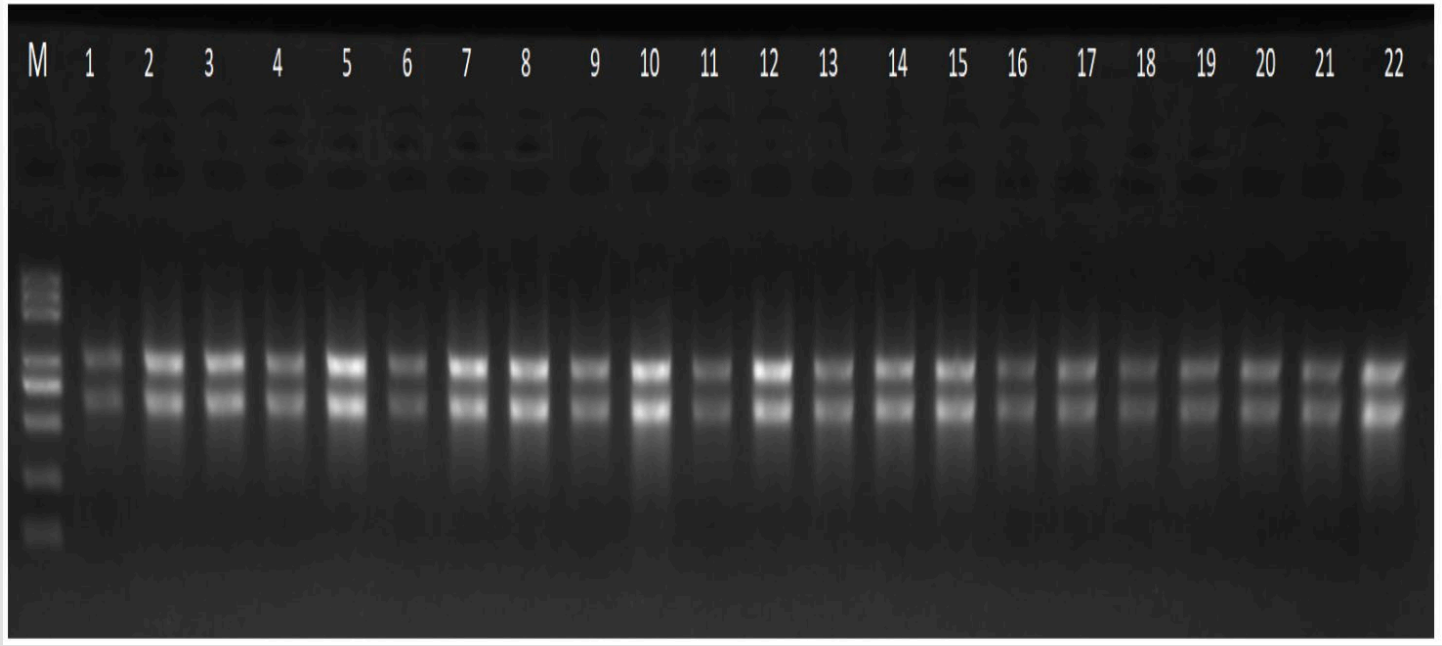
5 replicates/treatment

4 experimental runs

	Acetate, mmol/l	Propionate, mmol/l	Butyrate, mmol/l	Isovalerate, mmol/l	Valerate, mmol/l	Total VFA	total Lactic acid, g/l
LPS.F	22.25 ± 1.94	7.10 ± 1.24	4.24 ± 0.27	1.14 ± 0.06	0.93 ± 0.05	35.66 ± 3.46	0.02 ± 0.001
LPS.E	23.05 ± 0.65	7.07 ± 0.32	4.54 ± 0.21	1.20 ± 0.03	0.99 ± 0.04	36.84 ± 1.22	0.02 ± 0.002
LPS_R	29.02 ± 0.27	8.36 ± 0.60	4.20 ± 1.03	0.62 ± 0.09	0.53 ± 0.06	42.74 ± 2.03	0.02 ± 0.003

Sampling:

RIN> 8



6 h after inoculation:

- VFA, lactic acid and LPS
- 16S rRNA sequencing

24 h after inoculation:

- VFA, lactic acid and LPS
- 16S rRNA sequencing
- RNA sequencing

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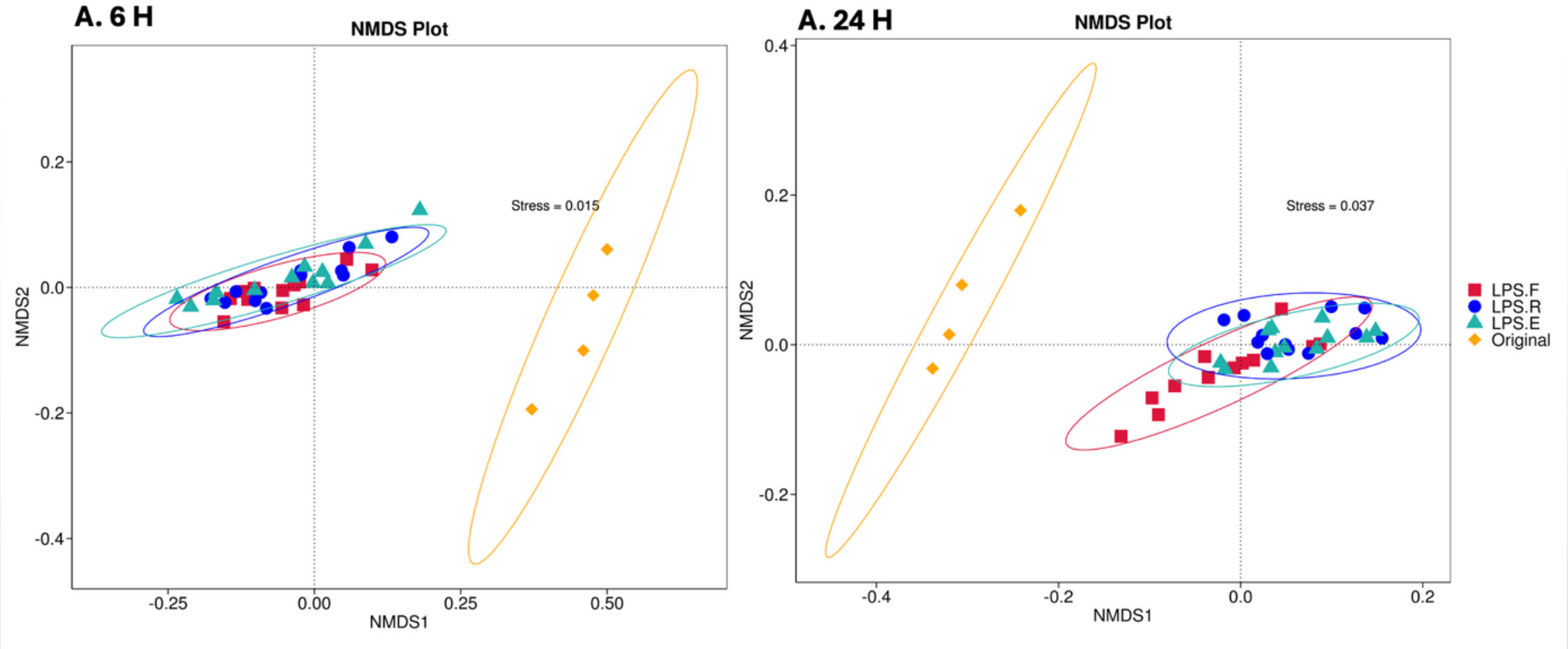
Results: Effects on the rumen fermentation profiles

	6 h after inoculation					24 h after inoculation				
Item[1]	LPS_F	LPS_E	LPS_R	S.E	P-value	LPS_F	LPS_E	LPS_R	S.E	P-value
pH	6.22	6.23	6.26	0.015	0.103	6.18b	6.20b	6.25a	0.012	<0.01
LPS, EU/ml	5,655	6,462	5,646	726	0.674	32,288b	41,927a	24,723c	12119	<0.01
Lactic acid, g/L	3.83c	3.62b	4.08a	0.122	<0.01	0.023b	0.033b	0.179a	0.042	<0.01
TVFA, mmol	35.9b	35.9b	43.1a	0.852	<0.01	61.2b	62.9b	69.3a	1.44	<0.01
Acetate, mmol	23.4b	23.5b	28.7a	0.428	<0.01	32.9b	33.4b	37.9a	0.842	<0.01
Propionate, mmol	8.15b	7.85b	9.42a	0.397	<0.01	22.8b	23.8b	25.8a	0.547	<0.01
Butyrate, mmol	3.64b	3.79b	4.50a	0.244	0.01	4.64b	4.82b	5.10a	0.143	<0.01
Valerate, mmol	0.72b	0.77a	0.40c	0.012	<0.01	0.82a	0.87a	0.54b	0.02	<0.01
Isovalerate, mmol	0.03a	0.03a	0.01b	0.009	<0.01	0.02a	0.02a	0.01b	6E-04	<0.01
Acetate, %	65.2b	65.5b	66.8a	0.639	0.01	53.8b	53.1c	54.7a	0.323	<0.01
Propionate, %	22.6a	21.8b	21.9b	0.677	<0.01	37.3b	37.9a	37.2b	0.436	0.05
Butyrate, %	10.2	10.5	10.4	0.468	0.79	7.58a	7.66a	7.36b	0.138	<0.01
Valerate, %	2.01b	2.14a	0.93c	0.042	<0.01	1.34a	1.38a	0.78b	0.018	<0.01

Results: Effects on the rumen bacteria



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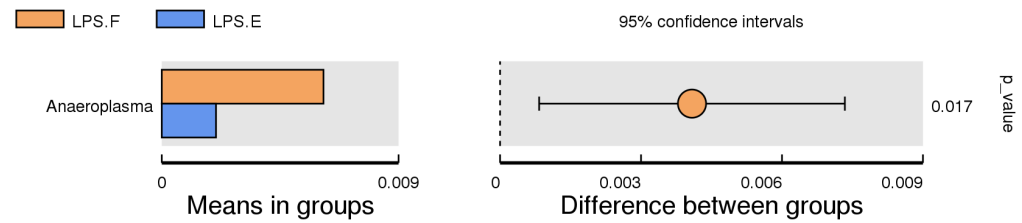
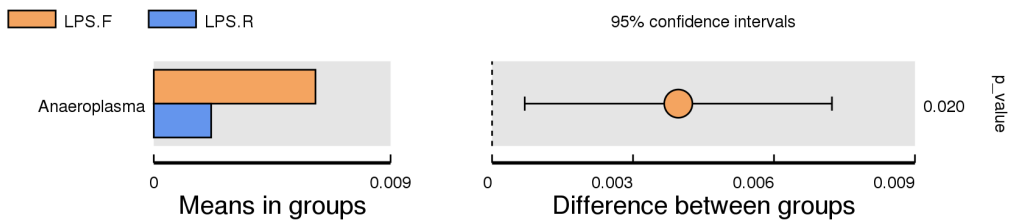
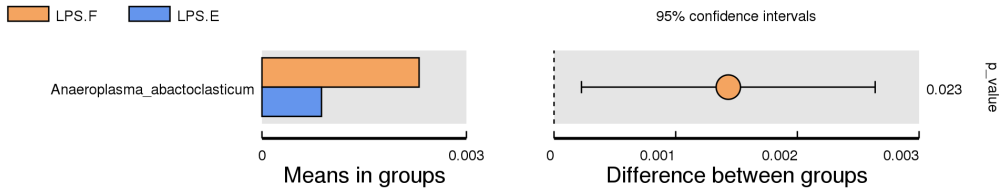
Results: Effects on the rumen bacteria composition (6 h after inoculation

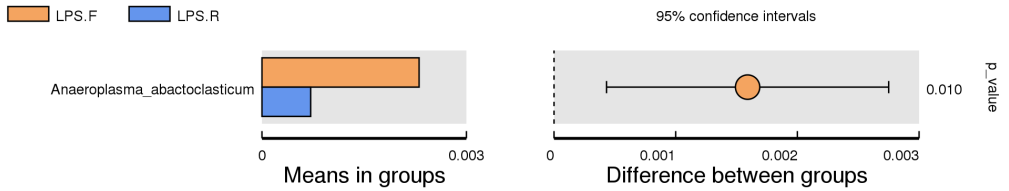
Anaeroplasma





Anaeroplasma_abactoclasticum





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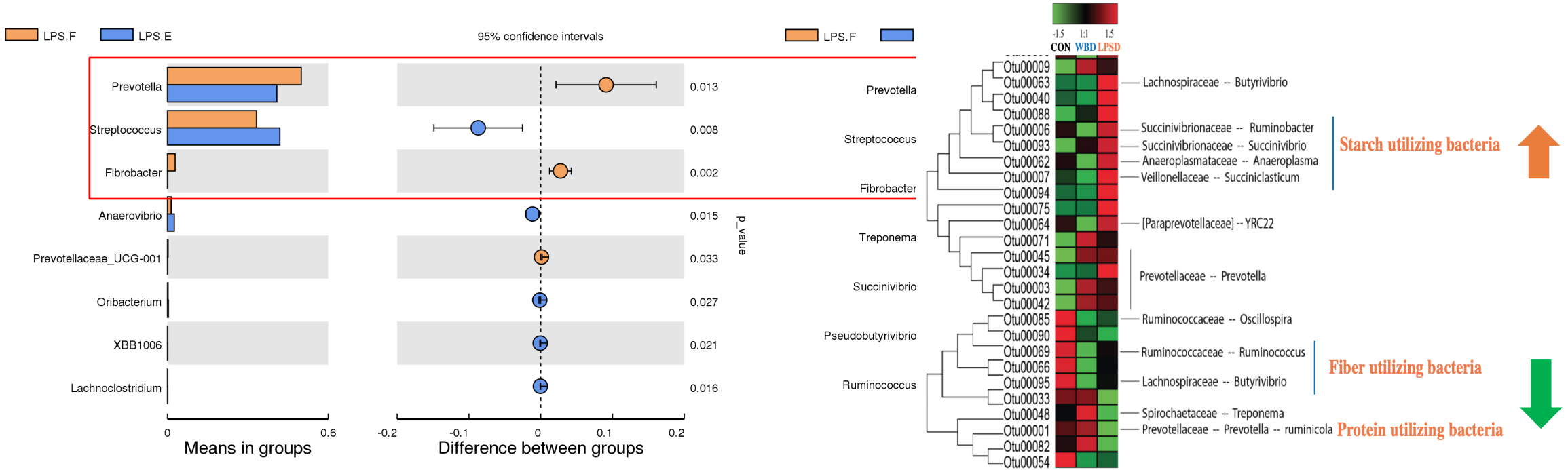
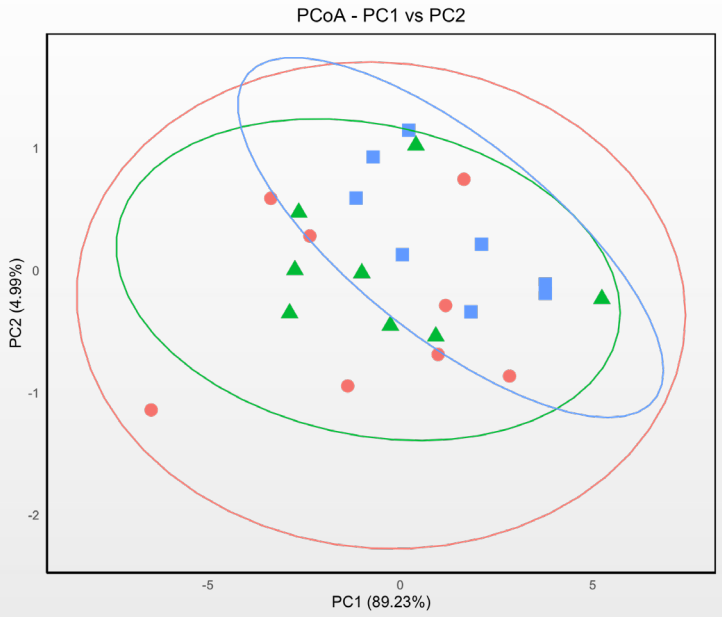
Results: Effects on the rumen bacteria composition (24 h after inoculation



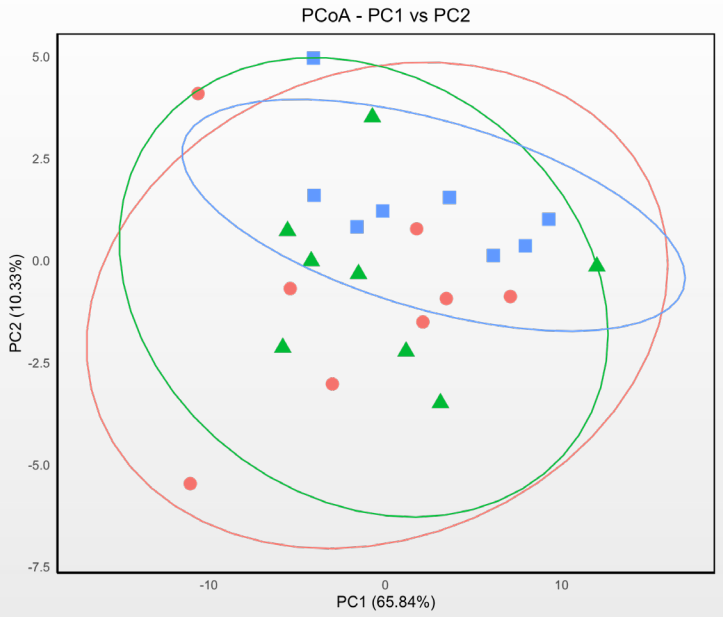
Results: Effects on functional pathways in rumen bacteria

By using the DIAMOND tool with the UniRef90 database (v. 201901b)

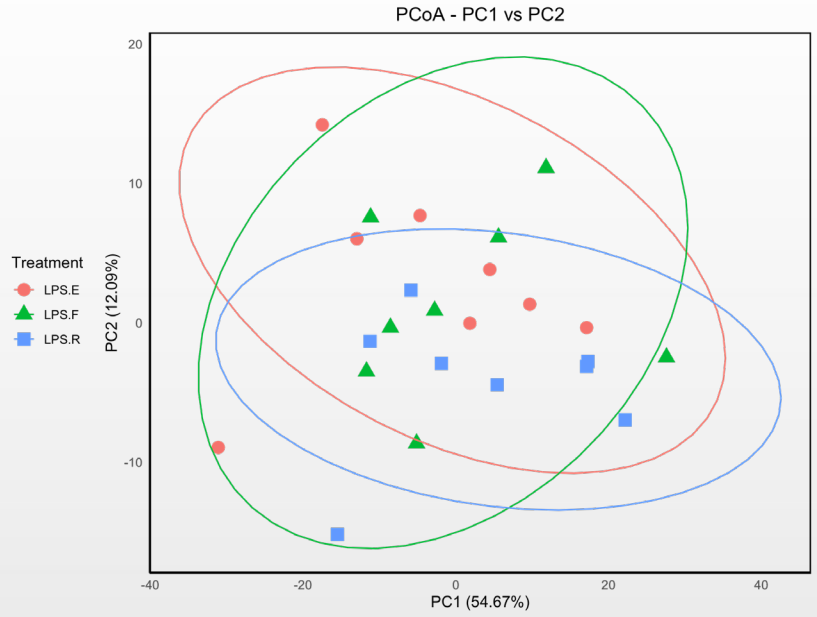
Level 1



Level 2



Level 3



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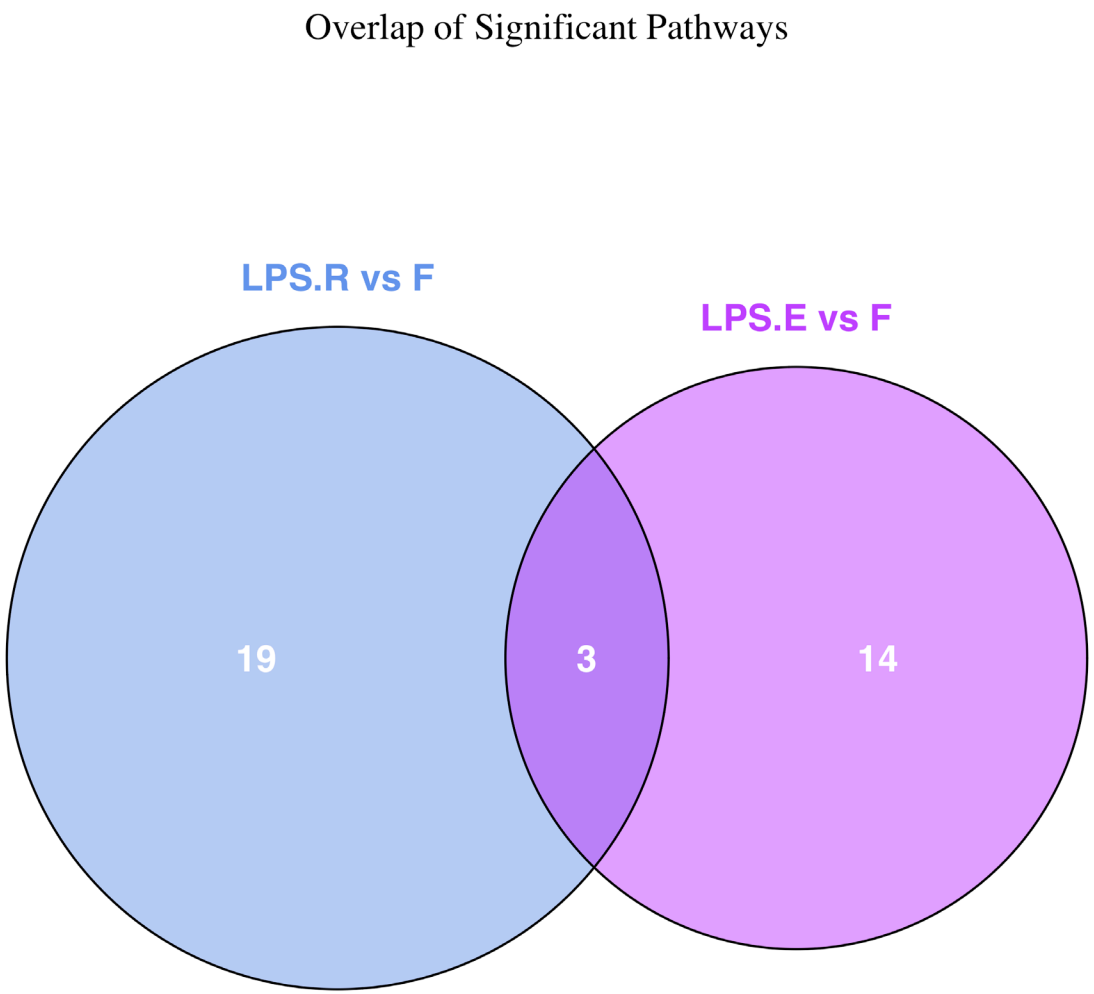
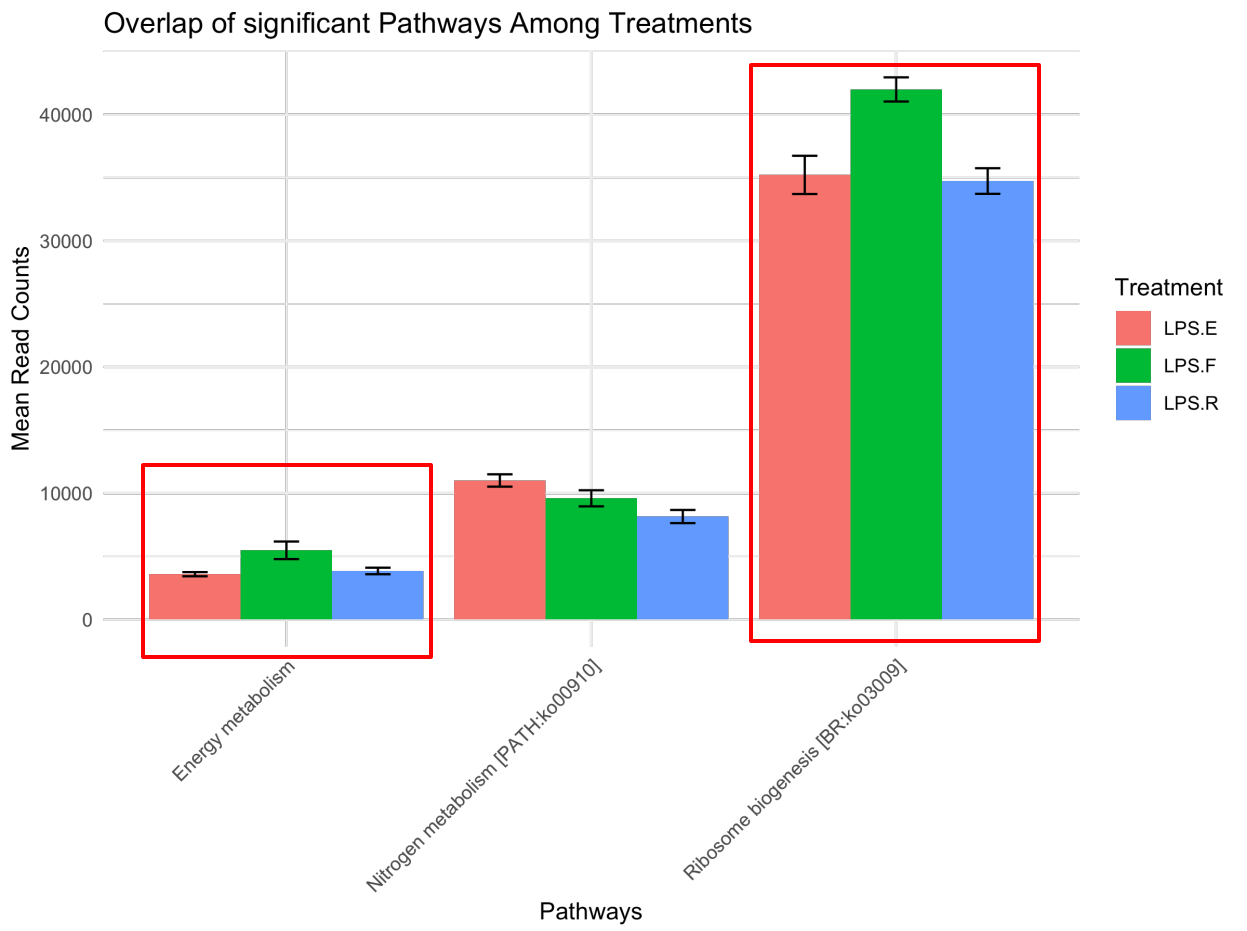
Results: Effects on functional pathways in rumen bacteria





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Results: Effects on functional pathways in rumen bacteria

LPS. R vs LPS.F

Pathways	logFC	FDR
DNA replication proteins [BR:ko03032]	-0.57	<0.01
DNA repair and recombination proteins [BR:ko03400]	-0.31	<0.01
Protein export [PATH:ko03060]	-0.34	<0.01
Bacterial secretion system [PATH:ko03070]	-0.34	<0.01
Peroxisome [PATH:ko04146]	-0.32	<0.01
Secretion system [BR:ko02044]	-0.26	<0.01
Quorum sensing [PATH:ko02024]	-0.24	<0.01
Transcription machinery [BR:ko03021]	-0.23	<0.01
Ribosome biogenesis [BR:ko03009]	-0.22	<0.01
Methane metabolism [PATH:ko00680]	-0.51	<0.01
Energy metabolism	-0.41	<0.01
Glycolysis / Gluconeogenesis [PATH:ko00010]	-0.38	0.01
RNA degradation [PATH:ko03018]	-0.50	0.01
Cofactor metabolism	0.36	0.01
Messenger RNA biogenesis [BR:ko03019]	-0.47	0.01
Drug metabolism - other enzymes [PATH:ko00983]	-0.29	0.01
Nitrogen metabolism [PATH:ko00910]	-0.19	0.02
Butanoate metabolism [PATH:ko00650]	-0.15	0.02
Carbohydrate metabolism	0.27	0.02
Non-homologous end-joining [PATH:ko03450]	0.31	0.04
Arachidonic acid metabolism [PATH:ko00590]	0.28	0.04
Membrane trafficking [BR:ko04131]	-0.25	0.04

LPS. E vs LPS.F

Pathways	logFC	FDR
Energy metabolism	-0.57	<0.01
Sesquiterpenoid and triterpenoid biosynthesis [PATH:ko00909]	-1.13	<0.01
kanamycin and gentamicin biosynthesis [PATH:ko00524]	0.32	<0.01
Neomycin	0.32	<0.01
Ribosome biogenesis [BR:ko03009]	-0.24	<0.01
Protein kinases [BR:ko01001]	0.26	<0.01
PPAR signaling pathway [PATH:ko03320]	-0.28	<0.01
Nitrogen metabolism [PATH:ko00910]	0.23	<0.01
Streptomycin biosynthesis [PATH:ko00521]	0.18	0.01
Flagellar assembly [PATH:ko02040]	-0.47	0.01
Galactose metabolism [PATH:ko00052]	0.21	0.01
Lipoarabinomannan (LAM) biosynthesis [PATH:ko00571]	-1.85	0.01
Proteasome [PATH:ko03050]	-4.94	0.02
Ribosome [BR:ko03011]	-0.34	0.02
Ribosome [PATH:ko03010]	-0.34	0.02
Biosynthesis of various secondary metabolites - part 2 [PATH:ko00998]	-0.46	0.02
Vitamin B6 metabolism [PATH:ko00750]	0.14	0.04

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Conclusion

- LPS from different sources consistently alter rumen bacterial communities, highlighting their critical role in the pathogenesis of Subacute Ruminal Acidosis (SARA).
- LPS derived from *E. coli* can be effectively used as a representative model to study the effects of LPS on rumen bacteria, providing valuable insights into microbial dynamics under SARA conditions.

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