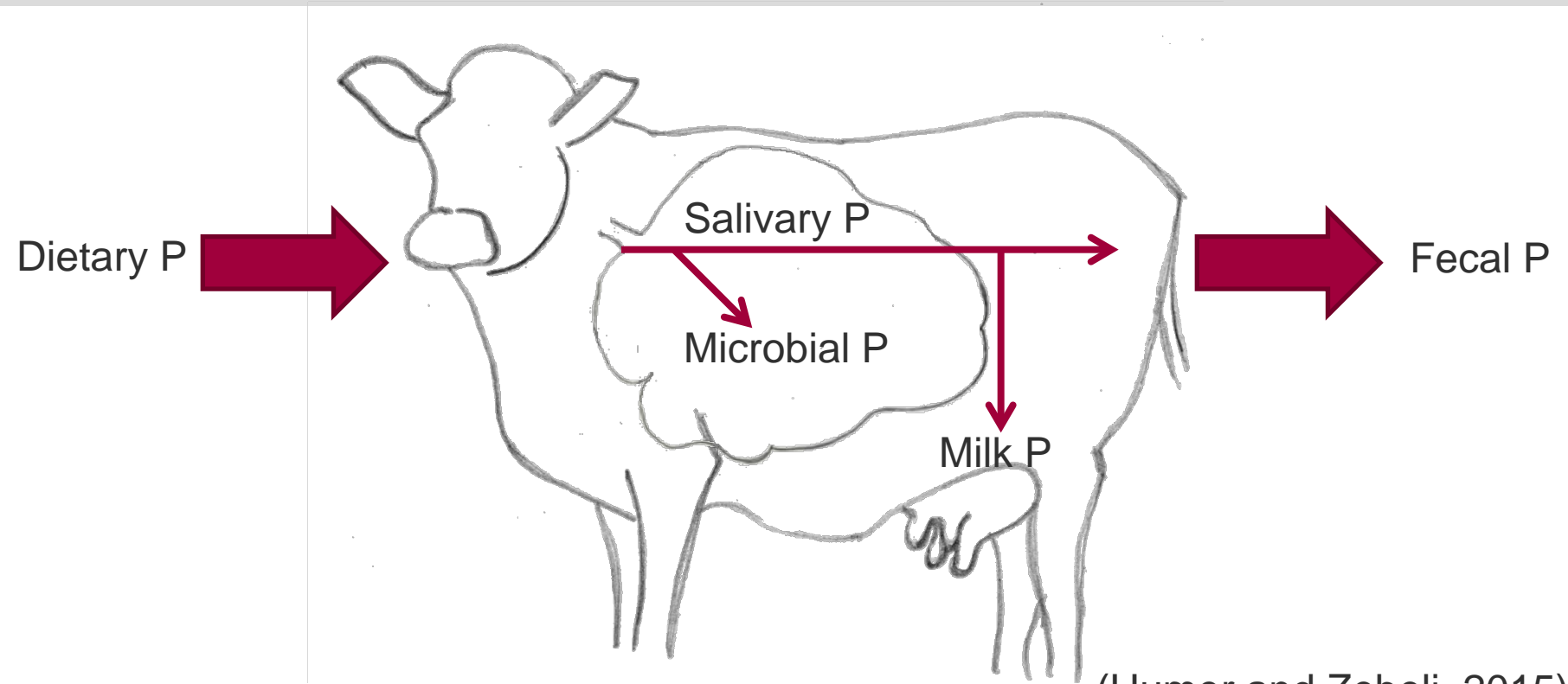# Effect of Lactic acid and P levels on rumen microbial dynamics

R.M. Petri, B.U. Metzler-Zebeli & Q. Zebeli
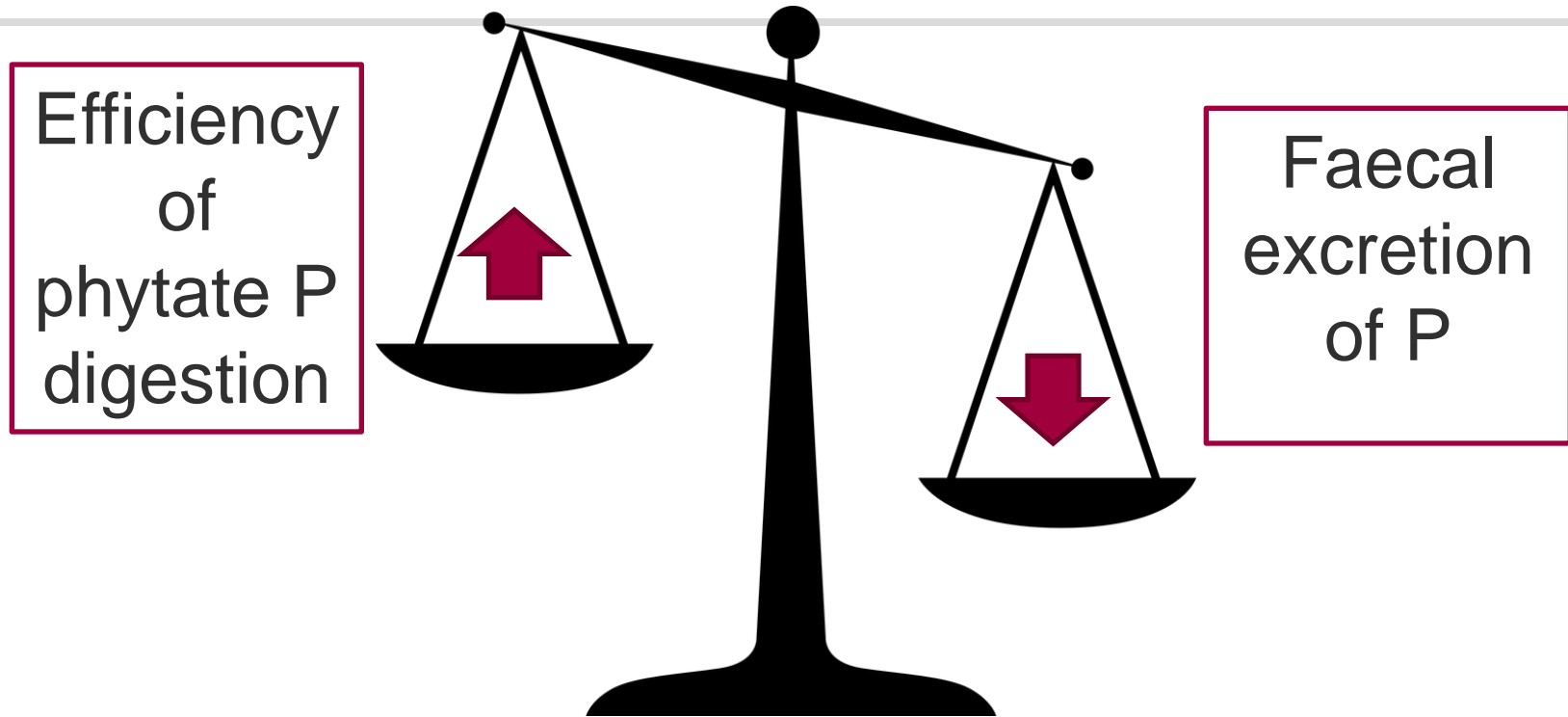
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Phosphorus Cycle in Ruminants
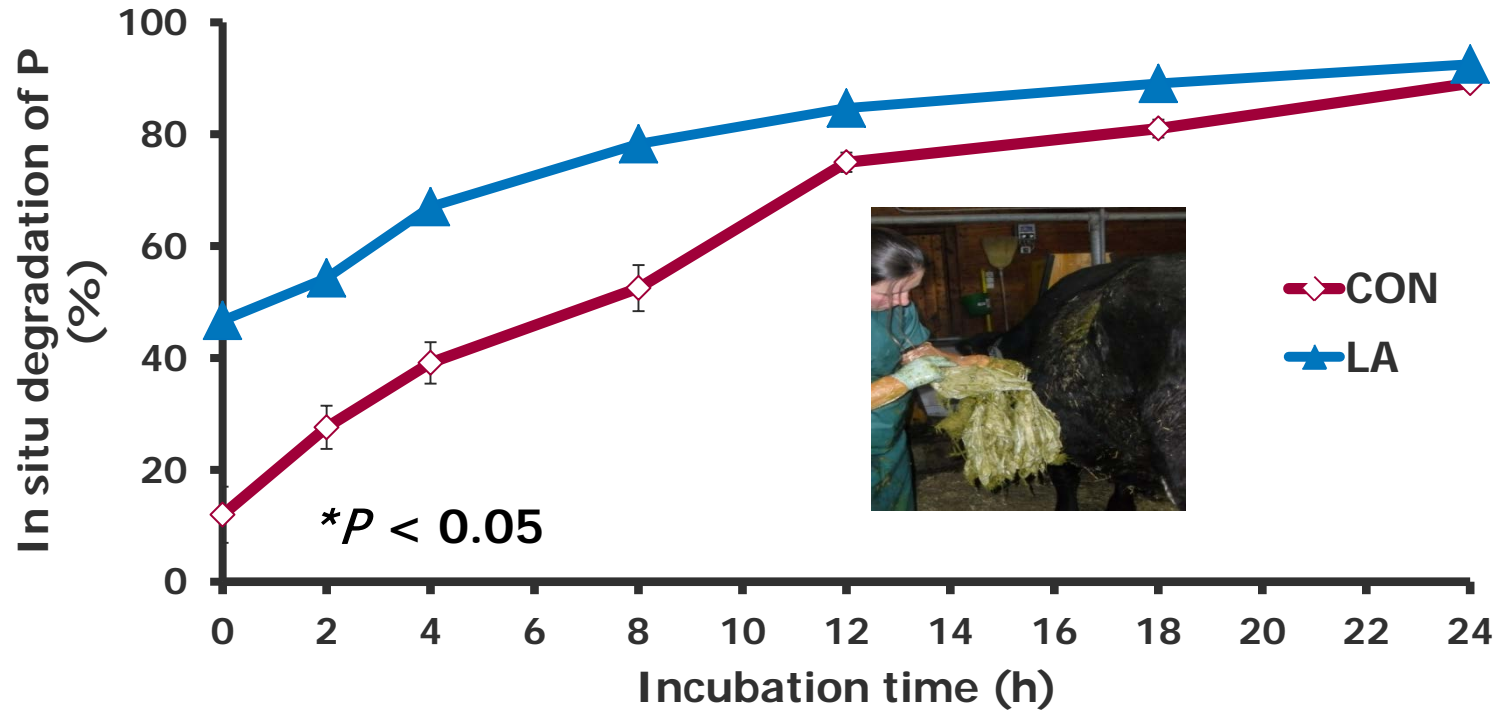

(Humer and Zebeli, 2015)

Improving P Availability

Efficiency of phytate P digestion

Faecal excretion of P

Brask/Pedersen et al. 2013; Jarret et al. 2014; Humer et al. 2015a

Degradation of Organic P +LA

Khol-Parisini et al. (2015)

Hypothesis

The treatment of concentrates with 5% lactic acid in early lactation diets will increase the availability of P to microbal degradation and decrease the faecal excretion of P

Feeding Experiment

- Lactating dairy cows (n=17)
- 4 primiparous; 13 multiparous
- Mixed breeds (Average BW 756 ± 159 kg)

Diets

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Ingredient, % of DM

Item	Dietary Treatment		
	CON	LA+P	LA-P
Meadow hay	10	10	10
Grass silage	10	10	10
Corn silage	33	33	33
Concentrate with inorganic P, without LA			
	47	-	-
Concentrate with inorganic P, with LA*			
	-	47	-
Concentrate without inorganic P, with LA*			
	-	-	47

Nutrient composition % of DM

Item	Dietary Treatment		
	CON	LA+P	LA-P
DM, % of fresh feed	43.1	43.1	42.7
Ash	7.11	7	6.97
CP	18.5	18.9	19.5
NDF	34.8	33.5	31.6
ADF	22.3	21.7	19.7
Ether extract	2.1	2.16	2.22
NFC4	37.5	38.4	39.8
Ca, g/kg DM	9.63	9.66	9.58
P, g/kg DM	4.53	4.64	4.13
NEL, MJ/kg DM	7.06	7.23	7.17

*soaked with 5% lactic acíd for 24h before feeding

Harder et al. (2015)

Sample Analysis

- 50 g faeces per cow were collected rectally on d 29 and stored at -20°C
    - Chemical Analysis, DNA extraction, qPCR, Sequencing
- Illumina MiSeq platform and stitched by Microsynth (Balgach, Switzerland).
- QIIME (Caporaso et al. 2010)
- PiCRUST analysis with KEGG (Langille et al. 2013)
- Proc Mixed Procedure of SAS (V.9.4)

Alpha Diversity - Results

Variable	Treatment			SEM	*P*-value of Contrasts	
	CON	LA P+	LA P-		CON vs. LA	P+ vs. P-
Chao1	6990	6598	6902	118.7	0.521	0.772
Simpson	0.996	0.994	0.995	0.0007	0.317	0.912
Shannon	9.635	9.440	9.518	0.0564	0.346	0.905

- 2,737,093 quality filtered sequences
- 9398 Total OTUs
- 152 OTUs > 0.001% of sequences
- 161,005 mean sequences per sample

OTU Significant Variation: Relative Abundance

OTU's	Treatment			SEM	P-value of Contrasts	
	CON	P+LA	P-LA		CON vs. LA	P+ vs. P-
OTU 4	0.0113	0.0088	0.0082	0.0010	**0.04**	0.16
OTU 6	0.0107	0.0080	0.0075	0.0010	**0.05**	0.20
OTU 47	0.0025	0.0054	0.0035	0.0009	**0.005**	0.45
OTU 54	0.0017	0.0054	0.0025	0.0011	**0.04**	0.31
OTU 66	0.0015	0.0028	0.0030	0.0005	**0.01**	0.10
OTU 73	0.0030	0.0012	0.0022	0.0005	**0.04**	0.91
OTU 88	0.0027	0.0016	0.0023	0.0003	**0.05**	0.55
OTU 105	0.0017	0.0024	0.0013	0.0003	0.55	**0.05**
OTU 123	0.0012	0.0016	0.0025	0.0004	**0.05**	**0.02**
OTU 127	0.0028	0.0016	0.0017	0.0004	**0.01**	**0.007**
OTU 138	0.0020	0.0010	0.0010	0.0003	**0.04**	0.26

OTU Classification

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OTUs	CON vs. LA (P-value)	P+ vs. P- (P-value)	Description NCBI	Percent Similarity	Clostridium Cluster based on NCBI	Description via Green Genes
OTU 4	**0.04**	0.16	*Fastidiosipila sanguinis strain CCUG 47711*	90%	iii	Ruminococcaceae fam.
OTU 6	**0.05**	0.20	*Thermotalea metallivorans strain B2-1*	86%	xi	Clostridiaceae family
OTU 47	**0.005**	0.45	*Alkalibaculum bacchi strain CP11*	89%	xv	Clostridiales order
OTU 54	**0.04**	0.31	*Saccharofermentans acetigenes strain P6*	90%	iii	Ruminococcaceae fam.
OTU 66	**0.01**	0.10	*Saccharofermentans acetigenes strain P6*	91%	iii	Ruminococcaceae fam.
OTU 73	**0.04**	0.91	*Gracilibacter thermotolerans strain JW/YJL-S1*	87%	i/ii/iii	Clostridiales order
OTU 88	**0.05**	0.55	*Sporobacter termitidis strain SYR*	94%	iv	Ruminococcaceae fam.
OTU 123	**0.05**	**0.02**	*Defluviitalea saccharophila strain LIND6LT2*	88%	iii	Ruminococcaceae fam.
OTU 127	**0.01**	**0.007**	*Peptococcus niger strain JCM 6506*	89%	n/a	RC4-4 genus
OTU 138	**0.04**	0.26	*Gracilibacter thermotolerans strain JW/YJL-S1*	87%	i/ii/iii	Clostridiales order

qPCR

Variable	Treatment			SEM	P-value	
	CON	**P+LA**	**P-LA**	**SEM**	**Treat**	**Phos**
Total Bacteria ($\log_{10}$ Gene Copies/g faeces)	10.90	10.84	10.78	0.034	0.565	0.528
Percent of Total Bacteria determined as bacterial groups or speices %)						
Genus *Prevotella*	2.97	3.28	3.04	0.094	0.394	0.680
Lactobacillus group	0.0001	0.0001	0.0002	0.000	0.591	0.210
Clostridium cluster I	0.004	0.004	0.009	0.002	0.570	0.203
Clostridium cluster IV	0.03	0.03	0.02	0.000	0.664	0.620
Clostridium cluster XIVa	3.96	3.82	3.68	0.082	0.602	0.602
Genus *Ruminicoccus*	6.52	6.03	6.02	0.164	0.249	0.541
Turicibacter spp.	0.0026	0.0023	0.0021	0.000	0.572	0.653
Streptococcus spp.	0.0012	0.0011	0.0008	0.000	0.107	0.080
Total Methanogen ($\log_{10}$ Gene Copies/mL)	0.05	0.05	0.04	0.005	0.102	0.079

KEGG Predictive Pathways Phosphorus Responding OTUs

		Control	LA +P	LA -P	SEM	Contrasts	
						CON vs. LA	P+ vs. P-
Environmental Information Processing							
Membrane Transport	Phosphotransferase system (PTS)	537	319	572	74.8		0.73
Signal Transduction	Phosphatidylinositol signaling system	316			47.3	0.17	0.64
Metabolism							
Carbohydrate Metabolism	Inositol phosphate metabolism	316	58	190		0.17	0.64
Carbohydrate Metabolism	Pentose phosphate pathway	2771	1100	1675	412	0.17	0.64
Energy Metabolism	Oxidative phosphorylation	3664	1170	2101	594	0.18	0.64
Lipid Metabolism	Glycerophospholipid metabolism	2251	116	1386	325		0.64
Metabolism of Amino Acids	Phosphonate and phosphinate metabolism	69	26	33	15	0.24	0.66

Faecal Phosphorus

(g/kg)	Treatment			SEM	P-Value Contrasts	
	CON	LA P+	LA P-		CON vs. LA	P+ vs. P-
Faecal P	10.3	10.5	9.0	0.27	0.36	0.03

Conclusions

- LA increases availability of organic P

- P was not a limiting factor for faecal microbiota

- The use of LA in early lactation diets is a viable option for reducing cost and P pollution

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