

Supplementary material

Association between Ruminal pH and Rumen Fatty Acids Concentrations of Holstein Cows during the First Half of Lactation

Georgios C. Kitkas ¹, Georgios E. Valergakis ², Maria Kritsepi-Konstantinou ³, Athanasios I. Gelasakis ⁴, Panagiotis D. Katsoulos ¹, Emmanouil Kalaitzakis ¹ and Nikolaos K. Panousis ^{1,*}

¹ Clinic for Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece

² Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, Box 393, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece

³ Diagnostic Laboratory, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece

⁴ Department of Animal Science, School of Animal Biosciences, Agricultural University of Athens, 11855 Athens, Greece

* Correspondence: panousis@vet.auth.gr; Tel.: +30-2310994501

Citation: Kitkas, G.C.; Valergakis, G.E.; Kritsepi-Konstantinou, M.; Gelasakis, A.I.; Katsoulos, P.D.; Kalaitzakis, E.; Panousis, N.K.

Association between Ruminal pH and Rumen Fatty Acids

Concentrations of Holstein Cows during the First Half of Lactation.

2022, 2, 382–389. <https://doi.org/10.3390/ruminants2040026>

Academic Editors: Brian J. Leury and Maria Luisa Dettori

Received: 24 June 2022

Accepted: 21 September 2022

Published: 26 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Figure S1. Box-plots of ruminal pH and body condition score during the three sampling occasions (30, 90 and 150 days-in-milk).

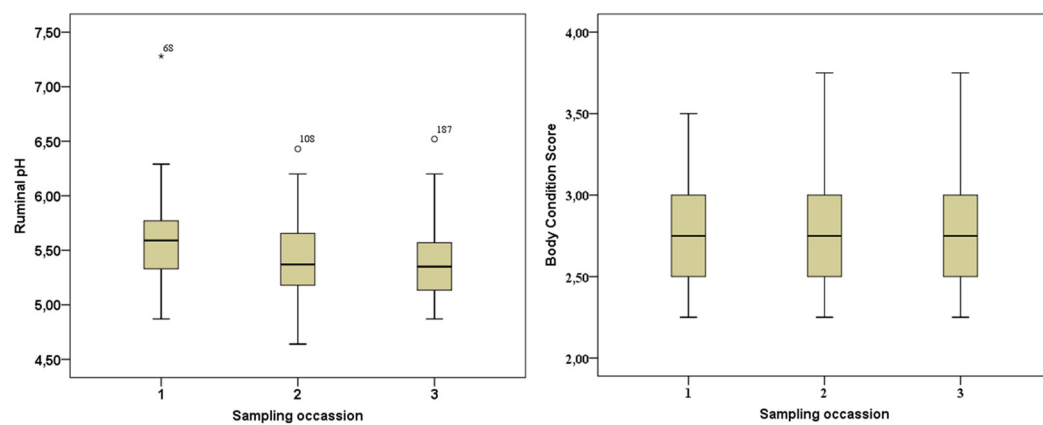


Figure S2. Box-plots of short and medium chain fatty acids content of ruminal fluid during the three sampling occasions (30, 90 and 150 days-in-milk).

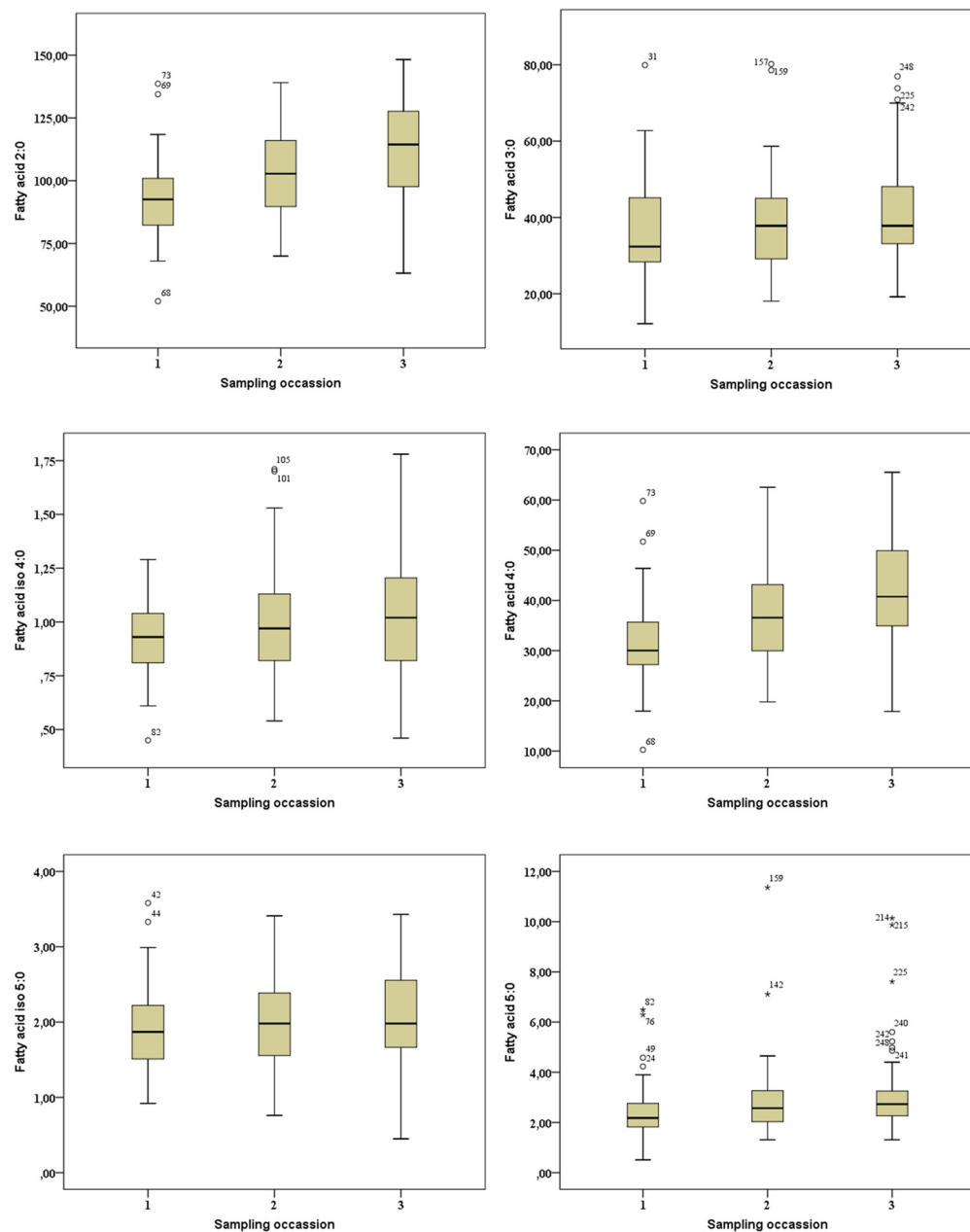


Figure S3. Box-plots of long chain fatty acids content of ruminal fluid during the three sampling occasions (30, 90 and 150 days-in-milk).

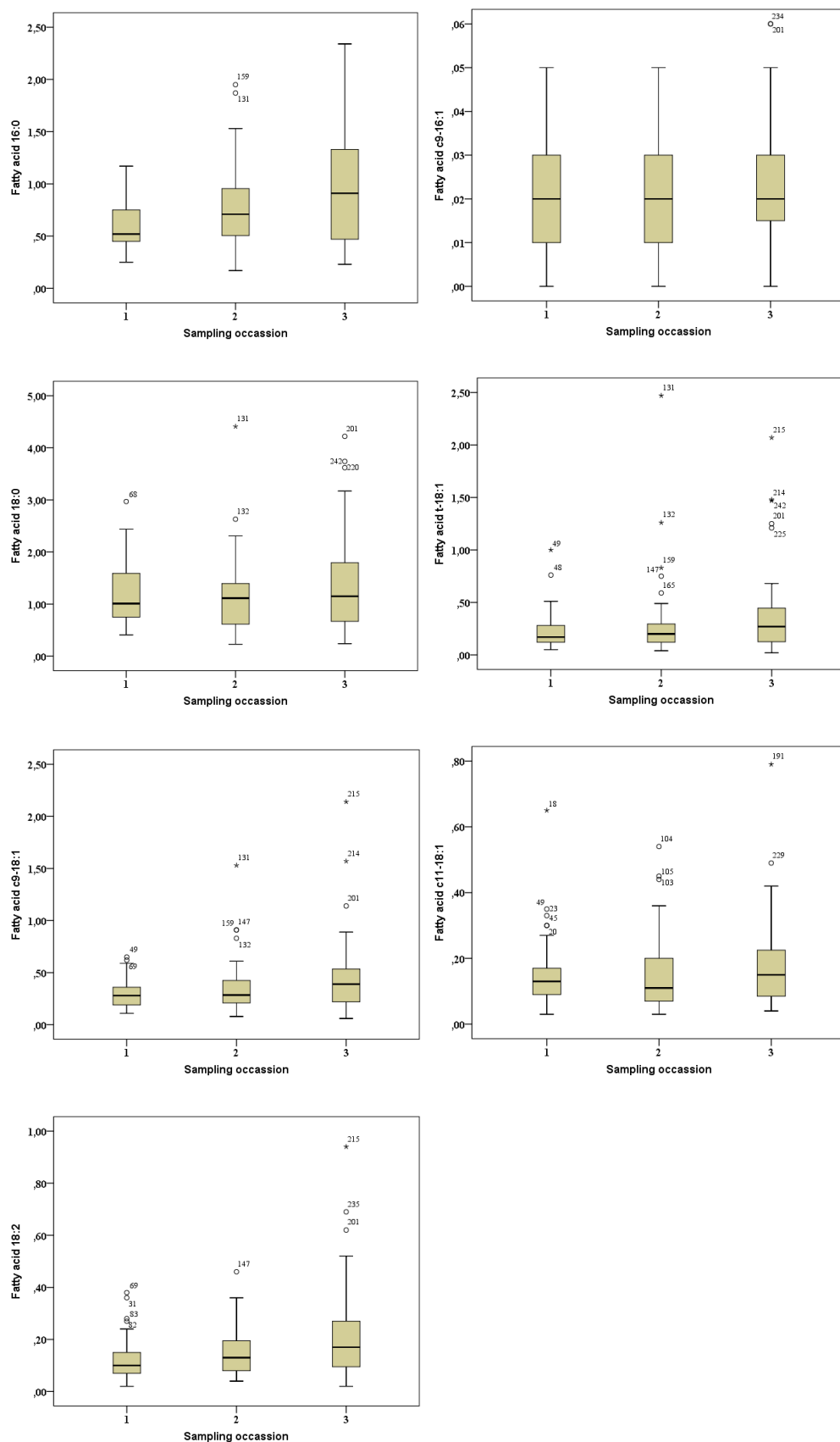


Table S1. Prevalence (number and %) of low ruminal pH status ($\text{pH} \leq 5.5$) cases at the three sampling points [30, 90 and 150 days in milk (DIM)] in relation to parity.

	30 DIM	90 DIM	150 DIM	Total
Primiparous (n=34)	20 (58.82%)	23 (67.65%)	24 (70.59%)	67
Multiparous (n=19)	4 (21.05%)	6 (31.58%)	11 (57.89%)	21
Total (n=53)	24 (45.28%)	29 (54.72%)	35 (66.04%)	88

Table S2. Associations between ruminal pH status and the rest of the parameters forced into model 1 with rumen content's fatty acids concentration in the studied cow population.

Fatty Acid	Parameter	Category level	B	SE	P-value	95 % CI	
						Lower	Upper
2:0	Ruminal pH status	Low	13.60	2.614	0.000	8.43	18.76
		Normal			"Ref"		
	Days post-calving	30	-17.14	2.695	0.000	-22.53	-11.74
		90	-8.75	3.390	0.012	-15.54	-1.97
		150			"Ref"		
	Parity number	1	-1.38	3.196	0.668	-7.78	5.02
		>1			"Ref"		
	Body Condition Score	Continuous	0.88	4.306	0.838	-7.65	9.42
	Intercept	Continuous	102.04	12.468	0.000	77.33	126.75
3:0	Ruminal pH status	Low	13.12	1.841	0.000	9.48	16.76
		Normal			"Ref"		
	Days post-calving	30	-2.52	2.033	0.218	-6.56	1.51
		90	-2.17	2.010	0.284	-6.15	1.82
		150			"Ref"		
	Parity number	1	2.73	2.131	0.207	-1.55	7.00
		>1			"Ref"		
	Body Condition Score	Continuous	1.50	2.972	0.615	-4.40	7.40
	Intercept	Continuous	27.90	8.573	0.002	10.88	44.91
iso 4:0	Ruminal pH status	Low	0.01	0.037	0.857	-0.07	0.08
		Normal			"Ref"		
	Days post-calving	30	-0.08	0.042	0.049	-0.17	-0.00
		90	-0.02	0.048	0.710	-0.11	0.08
		150			"Ref"		
	Parity number	1	-0.04	0.041	0.317	-0.12	0.04
		>1			"Ref"		
	Body Condition Score	Continuous	0.11	0.059	0.070	-0.01	0.22
	Intercept	Continuous	0.73	0.169	0.000	0.40	1.07
4:0	Ruminal pH status	Low	8.84	1.468	0.000	5.94	11.74
		Normal			"Ref"		
	Days post-calving	30	-8.97	1.631	0.000	-12.20	-5.74
		90	-3.57	1.612	0.029	-6.76	-0.37
		150			"Ref"		
	Parity number	1	0.87	1.672	0.603	-2.47	4.22
		>1			"Ref"		
	Body Condition Score	Continuous	-2.09	2.346	0.374	-6.75	2.56
	Intercept	Continuous	41.40	6.769	0.000	27.97	54.83

iso 5:0	Ruminal pH status	Low	0.18	0.099	0.073	-0.02	0.37
		Normal			"Ref"		
	Days post-calving	30	-0.15	0.105	0.161	-0.36	0.06
		90	-0.04	0.104	0.730	-0.24	0.17
		150			"Ref"		
	Parity number	1	0.05	0.129	0.722	-0.21	0.30
		>1			"Ref"		
	Body Condition Score	Continuous	-0.03	0.171	0.874	-0.37	0.31
	Intercept	Continuous	2.01	0.492	0.000	1.04	2.99

Table S2 continue a. Associations between ruminal pH status and the rest of the parameters forced into model 1 with rumen content's fatty acids concentration in the studied cow population.

Fatty Acid	Parameter	Category level	B	SE	P-value	95 % CI	
						Lower	Upper
5:0	Ruminal pH status	Low	0.61	0.174	0.001	0.27	0.96
		Normal			"Ref"		
	Days post-calving	30	-0.64	0.195	0.002	-1.03	-0.25
		90	-0.29	0.234	0.223	-0.76	0.18
		150			"Ref"		
	Parity number	1	0.48	0.290	0.105	-0.10	1.06
		>1			"Ref"		
	Body Condition Score	Continuous	-0.13	0.335	0.692	-0.80	0.53
	Intercept	Continuous	2.87	0.966	0.004	0.96	4.78
6:0	Ruminal pH status	Low	0.11	0.084	0.195	-0.06	0.28
		Normal			"Ref"		
	Days post-calving	30	-0.11	0.092	0.253	-0.29	0.08
		90	0.03	0.091	0.722	-0.15	0.21
		150			"Ref"		
	Parity number	1	-0.06	0.101	0.561	-0.26	0.14
		>1			"Ref"		
	Body Condition Score	Continuous	-0.05	0.138	0.710	-0.33	0.22
	Intercept	Continuous	1.07	0.399	0.008	0.28	1.87
7:0	Ruminal pH status	Low	-0.01	0.015	0.524	-0.04	0.02
		Normal			"Ref"		
	Days post-calving	30	0.03	0.019	0.095	-0.01	0.07
		90	0.03	0.017	0.104	-0.01	0.06
		150			"Ref"		
	Parity number	1	-0.01	0.016	0.507	-0.04	0.02
		>1			"Ref"		
	Body Condition Score	Continuous	-0.00	0.022	0.939	-0.05	0.04

16:0	Intercept	Continuous	0.05	0.066	0.426	-0.08	0.18
	Ruminal pH status	Low	0.15	0.051	0.004	0.05	0.25
		Normal			"Ref"		
	Days post-calving	30	-0.35	0.086	0.000	-0.52	-0.17
		90	-0.19	0.080	0.020	-0.35	-0.03
		150			"Ref"		
	Parity number	1	0.02	0.056	0.742	-0.09	0.13
		>1			"Ref"		
	Body Condition Score	Continuous	0.09	0.081	0.293	-0.08	0.25
	Intercept	Continuous	0.64	0.240	0.009	0.17	1.12
c9-16:1	Ruminal pH status	Low	0.00	0.002	0.470	-0.01	0.00
		Normal			"Ref"		
	Days post-calving	30	0.00	0.003	0.373	-0.01	0.00
		90	0.00	0.002	0.605	-0.01	0.00
		150			"Ref"		
	Parity number	1	0.00	0.002	0.299	0.00	0.01
		>1			"Ref"		
	Body Condition Score	Continuous	0.00	0.003	0.325	0.00	0.01
	Intercept	Continuous	0.01	0.009	0.118	0.00	0.03

Table S2 continue b. Associations between ruminal pH status and the rest of the parameters forced into model 1 with rumen content's fatty acids concentration in the studied cow population.

Fatty Acid	Parameter	Category level	B	SE	P-value	95 % CI	
						Lower	Upper
18:0	Ruminal pH status	Low	0.15	0.120	0.203	-0.08	0.39
		Normal			"Ref"		
	Days post-calving	30	-0.14	0.146	0.332	-0.43	0.15
		90	-0.22	0.143	0.127	-0.51	0.06
		150			"Ref"		
	Parity number	1	0.07	0.149	0.652	-0.23	0.37
		>1			"Ref"		
	Body Condition Score	Continuous	0.14	0.204	0.488	-0.26	0.55
	Intercept	Continuous	0.85	0.591	0.153	-0.32	2.02
t-18:1	Ruminal pH status	Low	0.04	0.034	0.203	-0.02	0.11
		Normal			"Ref"		
	Days post-calving	30	-0.14	0.047	0.006	-0.23	-0.04
		90	-0.08	0.044	0.068	-0.17	0.01
		150			"Ref"		
	Parity number	1	0.03	0.052	0.612	-0.08	0.13
		>1			"Ref"		

c9-18:1	Body Condition Score	Continuous	0.02	0.065	0.796	-0.11	0.15
	Intercept	Continuous	0.29	0.188	0.128	-0.08	0.66
	Ruminal pH status	Low	0.08	0.027	0.006	0.02	0.13
		Normal			"Ref"		
	Days post-calving	30	-0.13	0.045	0.006	-0.22	-0.04
		90	-0.08	0.039	0.043	-0.16	0.00
		150			"Ref"		
	Parity number	1	0.00	0.038	0.950	-0.07	0.078
		>1			"Ref"		
	Body Condition Score	Continuous	-0.03	0.050	0.572	-0.13	0.07
	Intercept	Continuous	0.47	0.148	0.002	0.18	0.77
c11-18:1	Ruminal pH status	Low	0.01	0.018	0.562	-0.03	0.05
		Normal			"Ref"		
	Days post-calving	30	-0.03	0.026	0.339	-0.08	0.03
		90	-0.02	0.026	0.343	-0.07	0.03
		150			"Ref"		
	Parity number	1	0.00	0.020	0.943	-0.04	0.04
		>1			"Ref"		
	Body Condition Score	Continuous	0.06	0.028	0.025	0.01	0.12
	Intercept	Continuous	0.00	0.083	0.975	-0.17	0.16
18:2	Ruminal pH status	Low	0.06	0.015	0.000	0.03	0.09
		Normal			"Ref"		
	Days post-calving	30	-0.08	0.024	0.002	-0.12	-0.03
		90	-0.06	0.026	0.028	-0.11	-0.01
		150			"Ref"		
	Parity number	1	0.02	0.016	0.196	-0.01	0.05
		>1			"Ref"		
	Body Condition Score	Continuous	-0.01	0.023	0.613	-0.06	0.03
	Intercept	Continuous	0.19	0.023	0.006	0.06	0.33

Table S3. Association between ruminal pH as a continuous variable and the rest of the parameters forced into model 1 with rumen content's fatty acids concentration in the studied cow population.

						95 % CI	
	Parameter	Category level	B	SE	P-value	Lower	Upper
2:0	Days post-calving	30	-14.21	2.36	0.000	-18.94	-9.48
		90	-8.08	3.09	0.011	-14.27	-1.89
		150			“Ref”		
	Parity number	1	-4.46	2.81	0.117	-10.08	1.15
		>1			“Ref”		
	Body Condition Score	Continuous	2.38	3.71	0.523	-4.97	9.73
pH	Continuous	-27.6	2.92	0.000	-33.38	-21.82	

	Intercept	Continuous	257.14	18.77	0.000	220.01	294.26
3:0	Days post-calving	30	-0.30	1.72	0.864	-3.70	3.11
		90	-1.78	1.68	0.290	-5.10	1.54
		150			"Ref"		
	Parity number	1	0.88	1.84	0.634	-2.81	4.57
		>1			"Ref"		
	Body Condition Score	Continuous	1.77	2.53	0.485	-3.24	6.79
	pH	Continuous	-23.98	2.07	0.000	-28.06	-19.89
	Intercept	Continuous	165.83	13.26	0.000	139.64	192.03
iso 4:0	Days post-calving	30	-0.08	0.042	0.059	-0.17	0.00
		90	-0.02	0.048	0.722	-0.11	0.08
		150			"Ref"		
	Parity number	1	0.05	0.042	0.285	-0.13	0.04
		>1			"Ref"		
	Body Condition Score	Continuous	0.11	0.059	0.069	-0.01	0.22
	pH	Continuous	-0.02	0.048	0.676	-0.11	0.07
	Intercept	Continuous	0.84	0.307	0.007	0.24	1.45
4:0	Days post-calving	30	-7.50	1.470	0.000	-10.42	-4.59
		90	-3.32	1.432	0.022	-6.16	-0.48
		150			"Ref"		
	Parity number	1	-0.33	1.538	0.829	-3.41	2.75
		>1			"Ref"		
	Body Condition Score	Continuous	-1.98	2.126	0.355	-6.19	2.24
	pH	Continuous	-16.00	1.755	0.000	-19.46	-12.53
	Intercept	Continuous	133.66	11.205	0.000	111.51	155.81
iso 5:0	Days post-calving	30	-0.10	0.105	0.328	-0.31	0.10
		90	-0.03	0.102	0.801	-0.23	0.18
		150			"Ref"		
	Parity number	1	0.01	0.127	0.966	-0.25	0.26
		>1			"Ref"		
	Body Condition Score	Continuous	-0.03	0.168	0.873	-0.36	0.31
	pH	Continuous	-0.40	0.130	0.003	-0.66	-0.14
	Intercept	Continuous	4.30	0.853	0.000	2.61	5.98

Table S3 continue a. Association between ruminal pH as a continuous variable and the rest of the parameters forced into model 1 with rumen content's fatty acids concentration in the studied cow population.

95% CI							
Parameter	Category level	B	SE	P-value	Lower	Upper	
5:0	Days post-calving	30	-0.54	0.191	0.007	-0.92	-0.16
		90	-0.27	0.225	0.235	-0.72	0.18
		150			“Ref”		
	Parity number	1	0.31	0.278	0.265	-0.24	0.87

		>1			"Ref"		
	Body Condition Score	Continuous	-0.15	0.321	0.641	-0.79	0.49
	pH	Continuous	-1.12	0.217	0.000	-1.55	-0.69
	Intercept	Continuous	9.44	1.520	0.000	6.43	12.45
6:0	Days post-calving	30	-0.04	0.100	0.672	1.78	4.49
		90	0.05	0.080	0.504	-0.11	0.21
		150			"Ref"		
	Parity number	1	-0.11	0.098	0.246	-0.31	0.08
		>1			"Ref"		
	Body Condition Score	Continuous	0.02	0.133	0.868	-0.24	0.29
	pH	Continuous	-0.40	0.106	0.000	-0.61	-0.19
	Intercept	Continuous	3.13	0.684	0.000	1.78	4.49
	Days post-calving	30	0.03	0.019	0.123	-0.01	0.07
		90	0.03	0.017	0.106	-0.01	0.06
7:0		150			"Ref"		
	Parity number	1	-0.01	0.016	0.567	-0.04	0.02
		>1			"Ref"		
	Body Condition Score	Continuous	-0.002	0.023	0.922	-0.05	0.04
	pH	Continuous	0.02	0.021	0.327	-0.02	0.06
	Intercept	Continuous	-0.06	0.129	0.631	-0.32	0.19
	Days post-calving	30	-0.36	0.084	0.000	-0.53	-0.19
		90	-0.20	0.085	0.021	-0.37	-0.03
		150			"Ref"		
	Parity number	1	0.04	0.063	0.481	-0.08	0.17
16:0		>1			"Ref"		
	Body Condition Score	Continuous	0.08	0.089	0.391	-0.10	0.25
	pH	Continuous	-0.10	0.070	0.179	-0.23	0.04
	Intercept	Continuous	1.26	0.45	0.006	0.36	2.16
	Days post-calving	30	-0.002	0.003	0.414	-0.008	0.003
		90	-0.001	0.002	0.642	-0.006	0.004
		150			"Ref"		
	Parity number	1	0.002	0.002	0.377	-0.002	0.006
		>1			"Ref"		
	Body Condition Score	Continuous	0.003	0.003	0.302	-0.003	0.009
c9-16:1	pH	Continuous	0.001	0.003	0.810	-0.005	0.006
	Intercept	Continuous	0.009	0.017	0.574	-0.024	0.043
	Days post-calving	30	-0.19	0.149	0.206	-0.49	0.11
		90	-0.24	0.142	0.093	-0.53	0.04
		150			"Ref"		
	Parity number	1	0.14	0.152	0.353	-0.16	0.45
		>1			"Ref"		
	Body Condition Score	Continuous	0.12	0.206	0.576	-0.29	0.52
18:0							

pH	Continuous	0.07	0.159	0.668	-0.25	0.38
Intercept	Continuous	0.61	1.03	0.552	-1.42	2.64

Table S3 continue b. Association between ruminal pH as a continuous variable and the rest of the parameters forced into model 1 with rumen content's fatty acids concentration in the studied cow population.

		95 % CI					
	Parameter	Category level	B	SE	P-value	Lower	Upper
t18:1	Days post-calving	30	-0.14	0.047	0.003	-0.24	-0.05
		90	-0.09	0.044	0.055	-0.17	0.00
		150			"Ref"		
	Parity number	1	0.04	0.054	0.448	-0.07	0.15
		>1			"Ref"		
	Body Condition Score	Continuous	0.02	0.066	0.818	-0.12	0.15
	pH	Continuous	-0.004	0.044	0.926	-0.09	0.08
c9-18:1	Intercept	Continuous	0.33	0.311	0.286	-0.28	0.95
	Days post-calving	30	-0.13	0.046	0.005	-0.23	-0.04
		90	-0.08	0.041	0.042	-0.17	-0.003
		150			"Ref"		
	Parity number	1	0.01	0.042	0.759	-0.07	0.10
		>1			"Ref"		
	Body Condition Score	Continuous	-0.02	0.053	0.650	-0.13	0.08
c11-18:1	pH	Continuous	-0.05	0.036	0.201	-0.12	0.03
	Intercept	Continuous	0.76	0.253	0.003	0.26	1.26
	Days post-calving	30	-0.02	0.026	0.365	-0.08	0.03
		90	-0.02	0.026	0.340	-0.08	0.03
		150			"Ref"		
	Parity number	1	-0.002	0.020	0.910	-0.04	0.04
		>1			"Ref"		
18:2	Body Condition Score	Continuous	0.06	0.028	0.025	0.01	0.12
	pH	Continuous	-0.02	0.023	0.474	-0.06	0.03
	Intercept	Continuous	0.09	0.148	0.525	-0.20	0.39
	Days post-calving	30	-0.07	0.023	0.005	-0.12	-0.02
		90	-0.06	0.025	0.025	-0.11	-0.01
		150			"Ref"		
	Parity number	1	0.01	0.016	0.368	-0.02	0.05
		>1			"Ref"		
	Body Condition Score	Continuous	-0.003	0.023	0.906	-0.05	0.04
	pH	Continuous	-0.09	0.019	0.000	-0.13	-0.06
	Intercept	Continuous	0.72	0.120	0.000	0.49	0.96