

Drinking warm water in a cold environment impairs growth performance and alters the gastrointestinal microbiota in growing calves

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Electronic Supplementary Material (ESM)

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Table S1. Environment temperature, wind speed and WCI index during the experiment period

Time	Temperature (°C)	Speed (km/h)	WCI
8:00			
Minimum	-28.1	0.372	-32.5
Maximum	-5.02	4.13	-3.08
Average	-14.2	1.62	-15.8
14:00			
Minimum	-20.2	2.77	-28.5
Maximum	4.00	10.1	1.12
Average	-4.65	5.87	-9.80

WCI = wind chill index

Table S2. Test results of animal drinking water quality

Item	Result
Chroma	colourless
Turbidity	undetected
Odour	–
Visible	–
Total hardness	253 mg/l
pH	7.55
Total dissolved solids	454 mg/l
Chloride	91.4 mg/l
Sulphate	75.0 mg/l
Total coliforms	undetected
Fluoride	0.400 mg/l
Cyanide	undetected
Total arsenic	undetected
Total mercury	2.0×10^{-4} mg/l
Lead	5.8×10^{-3} mg/l
Hexavalent chromium	undetected
Cadmium	undetected
Nitrate	11.3 mg/l

Table S3. Effects of drinking water temperature on serum immune indexes of calves

Item	Treatment			SEM	P-value
	FC	FM	FW		
IgA (µg/ml)					
0 d	27.1	25.9	26.5	2.55	0.951
15 d	32.9	29.9	28.6	2.29	0.412
30 d	35.1	37.1	35.5	2.18	0.796
IgG (µg/ml)					
0 d	238	238	210	13.7	0.340
15 d	264	235	248	13.8	0.366
30 d	270	247	284	13.2	0.175
IL-1β (pg/ml)					
0 d	168	165	154	11.5	0.710
15 d	151	152	157	10.5	0.908
30 d	152	140	140	10.6	0.651
IL-6 (pg/ml)					
0 d	225	235	221	10.2	0.680
15 d	207	234	209	11.4	0.199
30 d	205	205	196	9.06	0.734
TNF-α (pg/ml)					
0 d	136	154	142	6.61	0.192
15 d	124	125	138	6.43	0.235
30 d	113	119	118	8.97	0.905

d = experimental day; FC = 5 °C to 10 °C; FM = 20 °C to 25 °C; FW = 35 °C to 40 °C; SEM = standard error of the mean

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Table S4. Effects of drinking water temperature on growth-related hormones in serum of calves

Item	Treatment			SEM	P-value
	FC	FM	FW		
GH (ng/ml)					
0 d	34.5	32.3	33.0	1.88	0.727
15 d	42.4	36.3	37.85	2.20	0.161
30 d	45.6	44.0	45.55	2.68	0.895
GRF (ng/l)					
0 d	3.83	3.77	3.78	0.339	0.991
15 d	4.39	4.26	4.29	0.326	0.952
30 d	5.47	5.18	5.18	0.411	0.420
IGF-1 (ng/ml)					
0 d	29.4	26.2	30.5	2.01	0.386
15 d	36.6	34.5	34.0	2.74	0.782
30 d	41.5	35.6	37.5	2.49	0.265
SS (ng/ml)					
0 d	41.1	46.5	44.7	2.55	0.379
15 d	37.2	41.2	40.7	2.70	0.535
30 d	38.7	35.3	33.3	2.48	0.323

d = experimental day; FC = 5 °C to 10 °C; FM = 20 °C to 25 °C; FW = 35 °C to 40 °C; SEM = standard error of the mean

Table S5. Information on the sequencing depth of 16S rRNA genes in ruminal microbiota

Sample ID	Total read bases (bp)	Clean reads	Mean_length	Min_length	Max_length	Good's-coverage
LW22424	15 235 147	36 281	419	216	496	0.998
LW22425	18 460 633	44 264	417	235	514	0.997
LW22427	16 028 842	38 059	421	233	518	0.997
LW22429	14 271 535	33 809	422	252	530	0.998
LW22433	15 258 818	36 505	417	265	467	0.998
LW22437	24 481 111	58 549	418	234	505	0.998
LW22438	15 324 745	36 699	417	245	530	0.998
LW22440	34 441 040	83 096	414	235	502	0.996
LW22441	15 403 201	36 925	417	258	475	0.998
LW22444	14 573 929	34 606	421	264	466	0.997
LW310	16 063 722	38 354	418	235	467	0.997
LW311	15 797 506	37 731	418	219	518	0.998
LW312	16 220 960	38 787	418	271	502	0.997
LW315	14 760 830	35 210	419	271	442	0.998
LW316	34 106 724	81 390	419	210	523	0.997
Summation	–	670 265	–	–	–	–
Average	18 695 249	44 684	419	243	497	0.997

Table S6. Information on the sequencing depth of 16S rRNA genes in fecal microbiota

Sample ID	Total read bases (bp)	Clean reads	Mean_length	Min_length	Max_length	Good's-coverage
FB22424	18 130 584	43 976	412	260	488	0.994
FB22425	36 860 252	89 762	410	246	514	0.995
FB22427	15 636 969	37 891	412	203	501	0.995
FB22429	28 481 367	68 489	416	258	498	0.996
FB22433	27 351 403	66 317	412	220	522	0.994
FB22437	18 167 808	44 106	412	246	524	0.995
FB22438	16 512 020	40 087	412	265	498	0.995
FB22440	17 415 086	42 367	411	247	491	0.995
FB22441	18 838 182	45 750	412	261	508	0.995
FB22444	18 083 758	43 695	414	200	530	0.995
FB310	16 555 267	39 771	416	245	511	0.994
FB311	16 338 004	39 572	413	258	502	0.994
FB312	17 142 144	41 451	414	280	433	0.994
FB315	18 406 824	44 693	412	281	535	0.994
FB316	39 587 806	96 097	412	214	505	0.995
Summation	–	784 024	–	–	–	–
Average	21 567 164	52 268	413	246	504	0.995

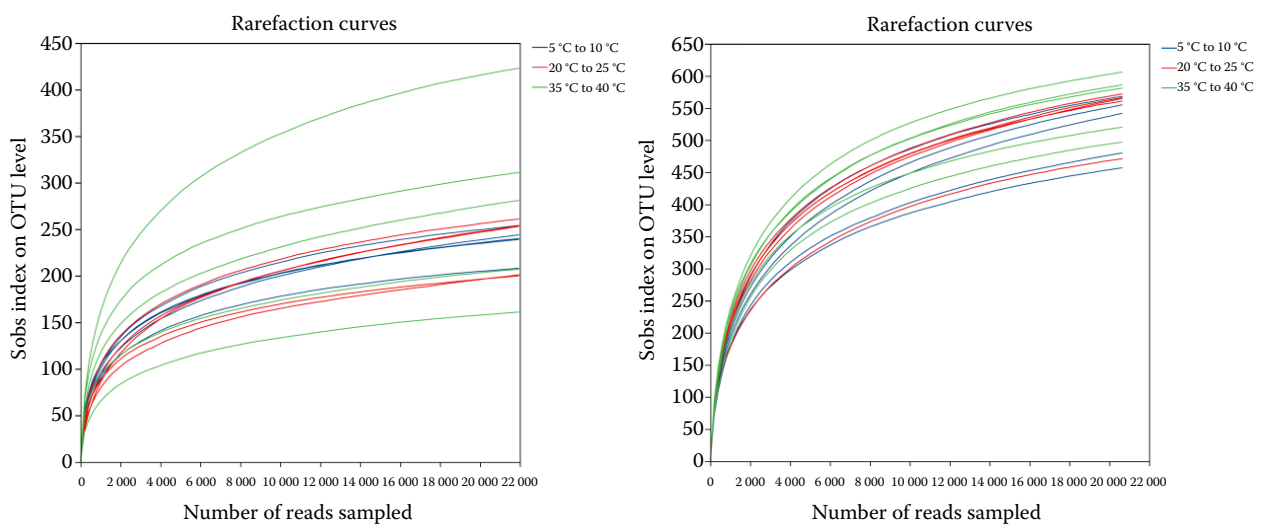


Figure S1. Effects of different drinking water temperatures on rarefaction curves of ruminal and fecal microbiota in weaned calves

(A) The ruminal microbiota; (B) The faecal microbiota

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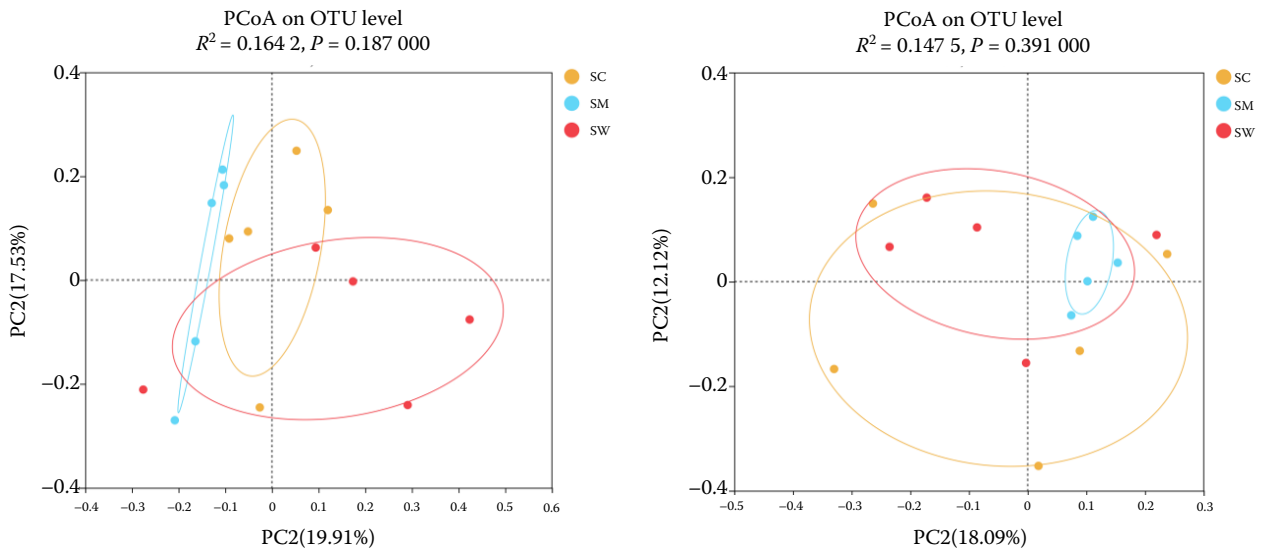


Figure S2. Effects of different drinking water temperatures on the beta diversity of ruminal and intestinal microbiota in weaned calves under experiment 2 conditions

(A) The PCoA plot of beta diversity indices of ruminal microbiota at the OTU level. (B) The PCoA plot of beta diversity indices of faecal microbiota at the OTU level