**Effects of the acid-base treatment of corn on rumen fermentation and microbiota, inflammatory response** **and growth performance in beef cattle fed high-concentrate diet**

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Short title: Acid-base treatment of corn for steers



**Supplementary Figure S1** Unweighted UniFrace principal coordinate analysis (PCoA) of rumen fluid bacterial community when beef cattle were fed different diets. PC1, PCoA axis 1; PC2, PCoA axis 2; LCD, low-concentrate diet based on corn steeped in tap water for 48 h; HCD, high-concentrate diet based on corn steeped in tap water for 48 h; HCDT, high-concentrate diet based on corn steeped in 1% (wt/wt) hydrochloric acid for 48 h in combination with subsequent sodium bicarbonate neutralization.

**Supplementary Table S1***Initial bacterial composition (%) in rumen fluid at the phylum level of each cattle (Rumen bacterial baseline)*

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Number1 | Bacteroidetes | Firmicutes | Proteobacteria | Tenericutes | Cyanobacteria | Spirochaetes | Fibrobacteres | Verrucomicrobia | TM7 | WPS-2 | Others |
| 1 | 58.32  | 33.29  | 5.67  | 0.67  | 1.07  | 0.30  | 0.01  | 0.24  | 0.04  | 0.00  | 0.39  |
| 2 | 56.15  | 35.30  | 6.26  | 0.54  | 0.95  | 0.24  | 0.02  | 0.10  | 0.11  | 0.00  | 0.33  |
| 3 | 54.03  | 37.66  | 4.35  | 0.50  | 0.02  | 0.36  | 0.70  | 1.90  | 0.02  | 0.01  | 0.44  |
| 4 | 53.02  | 40.17  | 4.44  | 0.74  | 0.43  | 0.21  | 0.04  | 0.00  | 0.03  | 0.02  | 0.90  |
| 5 | 51.82  | 41.69  | 5.06  | 0.12  | 0.02  | 0.11  | 0.49  | 0.11  | 0.00  | 0.19  | 0.39  |
| 6 | 61.15  | 31.02  | 6.65  | 0.58  | 0.06  | 0.39  | 0.01  | 0.04  | 0.00  | 0.05  | 0.05  |
| 7 | 60.90  | 31.38  | 6.61  | 0.55  | 0.06  | 0.04  | 0.06  | 0.04  | 0.03  | 0.00  | 0.33  |
| 8 | 58.03  | 34.75  | 4.06  | 0.47  | 0.03  | 1.24  | 0.37  | 0.45  | 0.05  | 0.17  | 0.37  |
| 9 | 57.71  | 35.88  | 3.69  | 0.85  | 0.00  | 0.47  | 0.45  | 0.34  | 0.03  | 0.28  | 0.29  |
| 10 | 57.09  | 36.66  | 4.56  | 0.57  | 0.07  | 0.27  | 0.09  | 0.02  | 0.16  | 0.01  | 0.49  |
| 11 | 62.63  | 31.19  | 3.58  | 0.55  | 0.38  | 0.69  | 0.28  | 0.21  | 0.09  | 0.00  | 0.39  |
| 12 | 55.72  | 36.86  | 4.61  | 0.98  | 0.16  | 0.32  | 0.50  | 0.36  | 0.00  | 0.02  | 0.46  |
| 13 | 50.60  | 43.68  | 4.47  | 0.11  | 0.01  | 0.36  | 0.32  | 0.08  | 0.06  | 0.06  | 0.24  |
| 14 | 50.97  | 39.31  | 7.92  | 0.80  | 0.20  | 0.10  | 0.09  | 0.09  | 0.03  | 0.00  | 0.48  |
| 15 | 57.82  | 32.90  | 7.45  | 0.13  | 0.09  | 0.20  | 0.37  | 0.41  | 0.00  | 0.08  | 0.55  |
| 16 | 61.16  | 33.02  | 4.45  | 0.58  | 0.11  | 0.19  | 0.01  | 0.08  | 0.00  | 0.07  | 0.33  |
| 17 | 62.47  | 30.10  | 5.32  | 0.90  | 0.28  | 0.10  | 0.00  | 0.02  | 0.02  | 0.00  | 0.79  |
| 18 | 58.77  | 34.81  | 5.17  | 0.18  | 0.05  | 0.03  | 0.00  | 0.03  | 0.66  | 0.01  | 0.29  |

1 Number of the experimental cattle in the present research.

**Supplementary Table S2** *Initial bacterial composition (%) in rumen fluid at the genus level of each cattle (Rumen bacterial baseline)*

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Number1 | Prevotella | UnclassifiedClostridiales | Succiniclasticum | UnclassifiedRF16 | UnclassifiedBacteroidales | UnclassifiedRuminococcaceae | UnclassifiedS24-7 | UnclassifiedSuccinivibrionaceae | UnclassifiedLachnospiraceae | Ruminococcus | Others |
| 1 | 39.69  | 6.04  | 5.85  | 6.46  | 4.62  | 5.21  | 3.11  | 6.06  | 3.27  | 3.61  | 16.05  |
| 2 | 40.00  | 6.42  | 5.37  | 7.85  | 6.35  | 3.24  | 3.24  | 6.04  | 2.42  | 3.78  | 15.29  |
| 3 | 40.14  | 7.63  | 6.45  | 6.10  | 6.12  | 3.56  | 3.27  | 7.66  | 2.48  | 2.01  | 14.58  |
| 4 | 39.88  | 6.89  | 7.93  | 6.80  | 7.76  | 2.88  | 2.44  | 5.58  | 2.88  | 2.09  | 14.88  |
| 5 | 40.69  | 6.24  | 7.50  | 8.04  | 7.06  | 5.57  | 4.19  | 4.18  | 2.89  | 2.75  | 10.88  |
| 6 | 35.08  | 4.12  | 6.63  | 7.58  | 5.70  | 5.02  | 5.60  | 7.88  | 4.54  | 3.81  | 14.05  |
| 7 | 38.37  | 7.60  | 7.21  | 8.58  | 7.49  | 5.64  | 3.25  | 5.22  | 2.74  | 2.07  | 11.83  |
| 8 | 34.19  | 6.50  | 8.75  | 7.58  | 4.37  | 6.76  | 5.75  | 5.31  | 4.06  | 3.34  | 13.38  |
| 9 | 36.15  | 8.90  | 5.33  | 6.22  | 5.15  | 5.79  | 4.43  | 5.33  | 4.89  | 4.00  | 13.80  |
| 10 | 36.88  | 8.49  | 5.75  | 6.88  | 7.49  | 3.49  | 3.50  | 7.32  | 2.01  | 2.81  | 15.39  |
| 11 | 41.18  | 6.03  | 5.40  | 6.78  | 5.02  | 2.77  | 3.79  | 4.69  | 4.76  | 4.04  | 15.55  |
| 12 | 38.57  | 7.92  | 8.11  | 5.22  | 6.77  | 5.23  | 4.03  | 4.13  | 4.04  | 3.11  | 12.87  |
| 13 | 38.38  | 5.42  | 5.41  | 7.81  | 6.64  | 3.59  | 3.59  | 6.91  | 2.48  | 3.59  | 16.17  |
| 14 | 40.76  | 7.05  | 6.45  | 6.77  | 4.22  | 6.84  | 3.42  | 6.53  | 2.64  | 2.52  | 12.79  |
| 15 | 40.32  | 6.37  | 5.17  | 5.94  | 6.26  | 5.87  | 5.53  | 5.06  | 4.15  | 2.46  | 12.87  |
| 16 | 34.05  | 6.27  | 6.09  | 5.23  | 7.61  | 4.32  | 4.92  | 6.69  | 5.25  | 2.42  | 17.14  |
| 17 | 43.17  | 7.06  | 5.44  | 5.52  | 4.49  | 3.04  | 4.36  | 5.42  | 4.38  | 3.59  | 13.53  |
| 18 | 36.31  | 5.70  | 5.87  | 6.37  | 5.60  | 5.97  | 3.46  | 5.49  | 4.60  | 2.96  | 17.68  |

1 Number of the experimental cattle in the present research.

**Supplementary Table S3** *Phylum level composition (%) of rumen fluid bacterial community of beef cattle fed different diets*

|  |  |  |  |
| --- | --- | --- | --- |
| Phylum | Diet | SEM | *P* value |
| LCD1 | HCD | HCDT |
| Firmicutes | 43.57A | 56.50B | 47.11A | 1.724 | 0.001 |
| Bacteroidetes | 50.37Aa | 35.02B | 45.23Cb | 1.846 | <0.001 |
| Spirochaetes | 1.40 | 1.30 | 2.05 | 0.261 | 0.500 |
| Actinobacteria | 0.30Aa | 2.80B | 0.62b | 0.426 | 0.017 |
| Proteobacteria | 0.80 | 1.06 | 2.43 | 0.356 | 0.153 |
| Tenericutes | 1.22 | 0.82 | 0.49 | 0.205 | 0.382 |
| Fibrobacteres | 0.95 | 0.64 | 0.42 | 0.135 | 0.311 |
| Verrucomicrobia | 0.52 | 0.41 | 0.95 | 0.142 | 0.304 |
| TM7 | 0.52 | 0.53 | 0.08 | 0.122 | 0.271 |
| WPS-2 | 0.09 | 0.09 | 0.22 | 0.048 | 0.534 |
| Other | 0.09 | 0.60 | 0.26 | 0.211 | 0.620 |
| Cyanobacteria | 0.05a | 0.12Bb | 0.01A | 0.015 | 0.006 |
| Synergistetes | 0.05 | 0.02 | 0.04 | 0.008 | 0.262 |
| Lentisphaerae | 0.03 | 0.04 | 0.03 | 0.015 | 0.984 |
| SR1 | 0.02 | 0.03 | 0.04 | 0.009 | 0.843 |
| Fusobacteria | 0.01 | 0.04 | 0.01 | 0.007 | 0.110 |
| Elusimicrobia | 0.01 | 0.01 | 0.02 | 0.005 | 0.503 |

1 LCD, low-concentrate diet based on corn steeped in tap water for 48 h; HCD, high-concentrate diet based on corn steeped in tap water for 48 h; HCDT, high-concentrate diet based on corn steeped in 1% (wt/wt) hydrochloric acid for 48 h in combination with subsequent sodium bicarbonate neutralization.

A-C Means of the same row not sharing an uppercase letter differ (*P* < 0.01).

a-b Means of the same row not sharing a lowercase letter differ (*P* < 0.05).

**Supplementary Table S4** *Genus level composition (%) of rumen fluid bacterial community of beef cattle fed different diets*

|  |  |  |  |
| --- | --- | --- | --- |
| Genus | Diet | SEM | *P* value |
| LCD1 | HCD | HCDT |
| *Prevotella* | 31.17A | 16.88B | 19.46B | 1.679 | <0.001 |
| Unclassified Bacteroidales | 9.70A | 15.21B | 16.31B | 0.831 | <0.001 |
| *Succiniclasticum* | 8.50A | 15.25B | 14.26B | 0.890 | <0.001 |
| Unclassified Clostridiales | 10.09 | 9.50 | 8.91 | 0.357 | 0.445 |
| Unclassified Ruminococcaceae | 10.17A | 5.10B | 9.36A | 0.652 | <0.001 |
| *Ruminococcus* | 7.00 | 5.98 | 6.30 | 0.498 | 0.706 |
| *Treponema* | 0.62 | 0.66 | 0.87 | 0.057 | 0.179 |
| Unclassified S24-7 | 3.90AB | 5.64A | 2.76B | 0.460 | 0.026 |
| Unclassified Lachnospiraceae | 1.99ab | 3.09a | 1.69b | 0.253 | 0.046 |
| *Butyrivibrio* | 0.73Aa | 2.84B | 2.36b | 0.313 | 0.004 |
| Unclassified RF16 | 2.92 | 2.09 | 2.26 | 0.330 | 0.575 |
| Unclassified Coriobacteriaceae | 0.04 | 2.00 | 0.16 | 0.495 | 0.187 |
| *Sharpea* | 0.05 | 2.29 | 2.16 | 0.734 | 0.386 |
| Unclassified BS11 | 1.30 | 0.56 | 0.57 | 0.186 | 0.175 |
| Unclassified Succinivibrionaceae | 0.29 | 1.76 | 1.48 | 0.628 | 0.615 |
| *Clostridium* | 0.11 | 1.69 | 0.33 | 0.548 | 0.457 |
| Unclassified RF39 | 1.02 | 0.75 | 0.38 | 0.212 | 0.516 |
| CF231 | 1.38Aa | 0.13B | 0.57b | 0.174 | 0.002 |
| *Bulleidia* | 0.06 | 0.49 | 0.36 | 0.095 | 0.148 |
| *Fibrobacter* | 0.94 | 0.50 | 0.42 | 0.116 | 0.138 |
| *Moryella* | 0.62 | 0.64 | 0.72 | 0.068 | 0.851 |
| *Asteroleplasma* | 0.95 | 0.07 | 0.25 | 0.224 | 0.234 |
| Unclassified F16 | 0.52 | 0.53 | 0.08 | 0.122 | 0.271 |
| Unclassified Christensenellaceae | 0.52a | 0.17b | 0.39ab | 0.070 | 0.010 |
| Unclassified Bifidobacteriaceae | 0.01 | 0.82 | 0.27 | 0.199 | 0.232 |
| *Coprococcus* | 0.26 | 0.32 | 0.37 | 0.077 | 0.842 |
| Other | 0.09 | 0.60 | 0.26 | 0.211 | 0.621 |
| Unclassified Veillonellaceae | 0.32 | 0.25 | 0.36 | 0.072 | 0.852 |
| *Bifidobacterium* | 0.22 | 0.45 | 0.22 | 0.073 | 0.334 |
| Unclassified WCHB1-25 | 0.14 | 0.24 | 0.52 | 0.110 | 0.383 |
| Unclassified [Mogibacteriaceae] | 0.47a | 0.14b | 0.24ab | 0.056 | 0.028 |
| *Selenomonas* | 0.28 | 0.34 | 0.21 | 0.059 | 0.705 |
| *Megasphaera* | 0.05a | 0.12a | 0.46b | 0.072 | 0.048 |
| *Sphaerochaeta* | 0.12 | 0.09 | 0.65 | 0.133 | 0.177 |
| Unclassified RFP12 | 0.37 | 0.11 | 0.20 | 0.066 | 0.246 |
| Unclassified [Paraprevotellaceae] | 0.40a | 0.13b | 0.20ab | 0.047 | 0.034 |
| *RFN20* | 0.19 | 0.16 | 0.46 | 0.064 | 0.123 |
| *Oscillospira* | 0.25 | 0.18 | 0.25 | 0.035 | 0.662 |
| Unclassified GMD14H09 | 0.16 | 0.08 | 0.45 | 0.100 | 0.335 |
| *Anaerovibrio* | 0.15 | 0.19 | 0.23 | 0.042 | 0.739 |
| *Desulfovibrio* | 0.21 | 0.13 | 0.15 | 0.022 | 0.385 |
| *YRC22* | 0.11 | 0.11 | 0.21 | 0.038 | 0.538 |
| *BF311* | 0.20 | 0.14 | 0.12 | 0.040 | 0.736 |
| Unclassified WPS-2 | 0.09 | 0.08 | 0.21 | 0.048 | 0.482 |
| *Blautia* | 0.01 | 0.25 | 0.17 | 0.055 | 0.181 |
| *Anaeroplasma* | 0.17 | 0.05 | 0.07 | 0.030 | 0.194 |
| *Anaerostipes* | 0.13 | 0.04 | 0.09 | 0.025 | 0.344 |
| *Shuttleworthia* | 0.08 | 0.02 | 0.18 | 0.051 | 0.471 |
| *Pseudobutyrivibrio* | 0.06 | 0.09 | 0.09 | 0.019 | 0.821 |
| [Prevotella] | 0.07 | 0.07 | 0.03 | 0.017 | 0.517 |
| *Mogibacterium* | 0.09 | 0.03 | 0.04 | 0.016 | 0.372 |
| Unclassified Prevotellaceae | 0.15 | < 0.01 | < 0.01 | 0.039 | 0.211 |
| Unclassified Oxalobacteraceae | 0.05 | 0.06 | 0.06 | 0.017 | 0.976 |
| *Ruminobacter* | 0.01 | 0.05 | 0.10 | 0.020 | 0.211 |
| Unclassified Streptophyta | 0.05a | 0.09Ab | < 0.01Bc | 0.012 | 0.002 |
| *Succinivibrio* | < 0.01 | 0.01 | 0.13 | 0.029 | 0.161 |
| *p-75-a5* | 0.04 | 0.05 | 0.02 | 0.007 | 0.143 |
| Unclassified Clostridiaceae | 0.05 | 0.02 | 0.03 | 0.007 | 0.111 |
| Unclassified Victivallaceae | 0.03 | 0.04 | 0.03 | 0.015 | 0.979 |
| *Pyramidobacter* | 0.05 | 0.01 | 0.04 | 0.008 | 0.242 |
| Unclassified SR1 | 0.02 | 0.03 | 0.04 | 0.009 | 0.857 |
| Unclassified Rickettsiales | 0.02 | 0.04 | 0.02 | 0.011 | 0.731 |
| *Dialister* | < 0.01 | 0.07 | 0.01 | 0.024 | 0.469 |
| *Acidaminococcus* | < 0.01 | 0.03 | 0.06 | 0.014 | 0.304 |
| *L7A E11* | 0.04 | 0.02 | 0.01 | 0.007 | 0.396 |
| *Mitsuokella* | < 0.01 | 0.04 | 0.03 | 0.010 | 0.194 |
| Unclassified RF32 | 0.01 | 0.02 | 0.04 | 0.013 | 0.618 |
| Unclassified YS2 | 0.01 | 0.04 | 0.01 | 0.008 | 0.090 |
| *Pseudoramibacter Eubacterium* | < 0.01a | 0.01ab | 0.04b | 0.007 | 0.042 |
| Unclassified Elusimicrobiaceae | 0.01 | 0.01 | 0.03 | 0.006 | 0.388 |
| *Lactobacillus* | 0.04a | < 0.01b | < 0.01b | 0.008 | 0.043 |
| *Desulfobulbus* | 0.01 | 0.02 | 0.02 | 0.005 | 0.774 |
| Unclassified Burkholderiales | 0.02 | 0.01 | 0.01 | 0.004 | 0.423 |
| *Roseburia* | < 0.01 | 0.03 | 0.01 | 0.007 | 0.188 |
| Unclassified Sphaerochaetaceae | < 0.01 | 0.02 | 0.01 | 0.007 | 0.369 |
| Unclassified Mycoplasmataceae | 0.01 | 0.02 | < 0.01 | 0.004 | 0.311 |
| *Fusobacterium* | 0.01a | 0.02b | < 0.01a | 0.004 | 0.040 |
| Unclassified WCHB1-41 | 0.01 | < 0.01 | 0.02 | 0.005 | 0.153 |
| *Dorea* | 0.01 | < 0.01 | 0.02 | 0.005 | 0.429 |
| Unclassified Desulfovibrionaceae | 0.01 | 0.02 | < 0.01 | 0.004 | 0.267 |
| *Leuconostoc* | < 0.01A | 0.02Bb | < 0.01a | 0.003 | 0.020 |
| *Suttonella* | < 0.01 | 0.02 | < 0.01 | 0.006 | 0.126 |
| Unclassified p-2534-18B5 | 0.01 | < 0.01 | 0.02 | 0.005 | 0.385 |
| Unclassified Acholeplasmatales | 0.02 | < 0.01 | < 0.01 | 0.007 | 0.297 |
| Unclassified Actinomycetaceae | 0.01 | 0.01 | < 0.01 | 0.003 | 0.547 |
| Unclassified Lactobacillales | < 0.01 | 0.01 | 0.01 | 0.003 | 0.467 |
| Unclassified Leptotrichiaceae | 0.01 | 0.01 | < 0.01 | 0.005 | 0.630 |
| *Corynebacterium* | 0.01 | < 0.01 | < 0.01 | 0.003 | 0.335 |
| Unclassified Spirochaetaceae | < 0.01 | 0.01 | 0.01 | 0.003 | 0.391 |
| *Atopobium* | 0.01 | 0.01 | < 0.01 | 0.002 | 0.601 |
| *PSB-M-3* | < 0.01 | 0.01 | < 0.01 | 0.002 | 0.280 |
| *Cetobacterium* | < 0.01 | < 0.01 | 0.01 | 0.003 | 0.515 |
| [Ruminococcus] | < 0.01 | 0.01 | < 0.01 | 0.002 | 0.516 |
| *Streptococcus* | 0.01 | < 0.01 | < 0.01 | 0.003 | 0.734 |
| [Eubacterium] | < 0.01 | 0.01 | < 0.01 | 0.003 | 0.150 |
| *TG5* | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.815 |
| Unclassified 0319-6G20 | 0.01 | < 0.01 | < 0.01 | 0.003 | 0.180 |
| Unclassified Chitinophagaceae | 0.01 | < 0.01 | < 0.01 | 0.003 | 0.503 |
| *Lachnobacterium* | < 0.01 | 0.01 | < 0.01 | 0.003 | 0.427 |
| *Adlercreutzia* | < 0.01 | < 0.01 | < 0.01 | 0.002 | 0.579 |
| *Cupriavidus* | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.615 |
| *Campylobacter* | < 0.01 | 0.01 | < 0.01 | 0.001 | 0.181 |
| Unclassified Comamonadaceae | < 0.01 | 0.01 | < 0.01 | 0.001 | 0.172 |
| *Paludibacter* | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.321 |
| Unclassified R4-45B | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.427 |
| Unclassified Enterobacteriaceae | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.141 |
| Unclassified Acetobacteraceae | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.588 |
| *Microbacterium* | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.321 |
| Unclassified Peptostreptococcaceae | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.427 |
| *Syntrophococcus* | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.427 |
| Unclassified HA64 | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.427 |
| *Akkermansia* | < 0.01 | < 0.01 | < 0.01 | 0.001 | 0.321 |

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A-B Means of the same row not sharing an uppercase letter differ (*P* < 0.01).

a-c Means of the same row not sharing a lowercase letter differ (*P* < 0.05).