**ESI Table 1** Ingredients and chemical composition of the experimental diet.

| Item | Contents |
|---|---|
| Ingredient [% of dry matter (DM)] | |
| Corn silage | 34.59 |
| Alfalfa hay | 11.85 |
| Oat hay | 4.33 |
| Ground Corn grain | 17.73 |
| Flaked corn | 3.98 |
| Soybean meal | 10.46 |
| Canola meal | 2.90 |
| Sugar beet pulp | 0.56 |
| Whole cottonseed | 7.12 |
| Fat powder[2] | 1.33 |
| Mineral and vitamin mix[2] | 3.87 |
| Molasses | 1.28 |
| Chemical composition (% of DM)[3] | |
| DM, % as fed | 51.43 |
| Organic matter | 91.12 |
| Crude protein | 15.80 |
| Neutral detergent fiber | 33.85 |
| Acid detergent fiber | 16.70 |
| Ether extract | 4.96 |
| Starch | 25.69 |
| Net energy for lactation (Mcal/kg of DM)[4] | 1.66 |

[1] Bergafat, a saturated free fatty acid supplement (Berg+Schmidt GmbH & Co. KG, Hamburg, Germany)

[2] Formulated to contain (as-is basis) trace mineral mix, 0.95%; dry corn distillers grains with solubles, 44.9%; MgO (56% Mg), 7.8%; NaCl, 6.6%; vitamin ADE premix, 0.48%; limestone, 39.2%; and selenium premix, 0.07%. Ca, 14.3%; P, 0.38%; Mg, 4.57%; K, 0.46%; S, 0.39%; Zn, 1,218 mg/kg; Fe, 186 mg/ kg, Se, 6.93 mg/kg; Cu, 370 mg/kg; vitamin A, 272,000 IU/kg; vitamin D, 75,000 IU/kg; and vitamin E, 2,080 IU/kg

[3] Analyzed values

[4] Calculated according to NRC (2001).

**ESI Table 2** Chemical composition of the citrus flavonoids extract.

| Item | Contents |
|---|---|
| Chemical composition, % of dry matter (DM) | |
| DM, % as fed | 92.16 |
| Organic matter | 85.37 |
| Crude ash | 14.63 |
| Crude protein | 2.08 |
| Neutral detergent fiber | 5.24 |
| Total flavonoids | 56.83 |
| Naringin | 25.61 |
| Hesperidin | 12.89 |
| Neohesperidin | 1.22 |

**ESI Table 3** Species-specific primers for the quantification of selected rumen microbes using a real-time qPCR assay.

| Target bacterial species | | Primer sequence (5'- 3') | Tm (°C) | Reference |
|---|---|---|---|---|
| Total bacteria | F | CGGCAACGAGCGCAACCC | 60 | [1] |
| | R | CCATTGTAGCACGTGTGTAGCC | | |
| Fungi | F | GAGGAAGTAAAAGTCGTAACAAGGTTTC | 58 | [1] |
| | R | CAAATTCACAAAGGGTAGGATGATT | | |
| Methanogens | F | TTCGGTGGATCDCARAGRGC | 58 | [2] |
| | R | GBARGTCGWAWCCGTAGAATCC | | |
| Protozoa | F | GCTTTCGWTGGTAGTGTATT | 55 | [3] |
| | R | CTTGCCCTCYAATCGTWCT | | |
| *Fibrobacter succinogenes* | F | GCGGGTAGCAAACAGGATTAGA | 60 | [4] |
| | R | CCCCCGGACACCCAGTAT | | |
| *Megaspheara elsdenii* | F | AGATGGGGACAACAGCTGGA | 60 | [4] |
| | R | CGAAAGCTCCGAAGAGCCT | | |
| *Prevotella brevis* | F | GGTTTCCTTGAGTGTATTCGACGTC | 64 | [5] |
| | R | CTTTCGCTTGGCCGCTG | | |
| *Prevotella ruminicola* | F | GAAAGTCGGATTAATGCTCTATGTTG | 63 | [5] |
| | R | CATCCTATAGCGGTAAACCTTTGG | | |
| *Ruminococcus albus* | F | ACGTCRTCCMCACCTTCCTC | 62 | [6] |
| | R | CCTCCTTGCGGTTAGAACA | | |
| *Ruminococcus flavefaciens* | F | CGAACGGAGATAATTTGAGTTTACTTAGG | 60 | [1] |
| | R | CGGTCTCTGTATGTTATGAGGTATTACC | | |
| *Ruminobacter amylophilus* | F | CTGGGGAGCTGCCTGAATG | 65 | [5] |
| | R | GCATCTGAATGCGACTGGTTG | | |
| *Selenomonas ruminantium* | F | CAATAAGCATTCCGCCTGGG | 57 | [4] |
| | R | TTCACTCAATGTCAAGCCCTGG | | |
| *Succinimonas amylolytica* | F | CGTTGGGCGGTCATTTGAAAC | 60 | [4] |
| | R | CCTGAGCGTCAGTTACTATCCAGA | | |
| *Streptococcus bovis* | F | TTCCTAGAGATAGGAAGTTTCTTCGG | 57 | [4] |
| | R | ATGATGGCAACTAACAATAGGGGT | | |

1. S.E. Denman and C.S. Mcsweeney, Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. *FEMS Microbiol. Ecol.*, 2006, **58**, 572-582.

2. S.E. Denman, N.W. Tomkins and C.S. Mcsweeney. Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiol. Ecol.*, 2007, **62**, 313-322.

3. J.T. Sylvester, S.K.R. Karnati, Z.Yu, M. Mark and J.L. Firkins. Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. *J. Nutr.*, 2004, **134**, 3378-3384.

4. M.K. Abdelmegeid, A.A. Elolimy, Z. Zhou, V. Lopreiato, J.C. McCann and J.J. Loor. Rumen-protected methionine during the peripartal period in dairy cows and its effects on abundance of major species of ruminal bacteria. *J. Anim. Sci. Biotechnol.*, 2018, **9**, 17.

5. D.M. Stevenson and P.J. Weimer, Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. *Appl. Microbiol. Biotechnol.*, 2007, **75**, 165-174.

6. S. Koike and Y. Kobayashi. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococus albus* and *Ruminococcus flavefaciens*. *FEMS Microbiol. Lett*., 2001, **204**, 361-366.

**ESI Table 4** Summary of metagenomic sequencing data.

| Sample | Raw reads | Optimized reads | Percent of raw reads | Contigs | N50 (bp) |
|---|---|---|---|---|---|
| CON_1 | 49,677,270 | 34,088,562 | 68.62 | 445,245 | 525 |
| CON_2 | 43,161,290 | 34,291,528 | 79.45 | 401,164 | 581 |
| CON_2 | 50,427,072 | 36,077,282 | 71.54 | 457,527 | 533 |
| CON_4 | 48,306,428 | 36,771,962 | 76.12 | 433,171 | 560 |
| CON_5 | 41,236,714 | 30,299,680 | 73.48 | 324,072 | 565 |
| CON_6 | 49,782,070 | 36,254,122 | 72.83 | 428,315 | 554 |
| CON_7 | 57,302,950 | 35,390,012 | 61.76 | 463,203 | 529 |
| CON_8 | 49,961,716 | 33,857,956 | 67.77 | 427,535 | 538 |
| CFE150_1 | 50,455,258 | 37,807,758 | 74.93 | 443,582 | 554 |
| CFE150_2 | 47,832,736 | 35,130,356 | 73.44 | 452,453 | 541 |
| CFE150_3 | 45,971,662 | 34,650,686 | 75.37 | 398,079 | 556 |
| CFE150_4 | 54,807,488 | 39,758,920 | 72.54 | 532,472 | 540 |
| CFE150_5 | 43,147,878 | 31,306,688 | 72.56 | 380,123 | 532 |
| CFE150_6 | 44,796,354 | 33,714,152 | 75.26 | 372,454 | 557 |
| CFE150_7 | 43,460,432 | 33,981,592 | 78.19 | 376,134 | 562 |
| CFE150_8 | 42,462,330 | 34,252,384 | 80.67 | 360,894 | 581 |
| Total | 762,789,648 | 557,633,640 | - | 6,696,423 | - |
| Mean | 47,674,353 | 34,852,103 | 73.41 | 418,526 | 551 |
| SD | 4,556,442 | 2,279,841 | 4.65 | 50,161 | 17 |

**ESI Table 5** PERMANOVA (permutational multivariate analysis of variance) of four microbial domains between CON and CFE150 samples**.**

| Taxonomy | SumsOfSqs | MeanSqs | F. Model | $R^2$ | *P*. adjust |
|---|---|---|---|---|---|
| Bacteria | 0.029 | 0.029 | 13.525 | 0.491 | 0.001 |
| Eukaryota | 0.023 | 0.023 | 1.900 | 0.120 | 0.192 |
| Archaea | 0.015 | 0.015 | 2.801 | 0.167 | 0.098 |
| Viruses | 0.064 | 0.064 | 29.825 | 0.681 | 0.001 |

**ESI Table 6** The significantly differential carbohydrate-active enzymes (>0.5% in at least one group) between the rumen fluid of dairy cows received the control diet (CON) and 150 g/d of citrus flavonoids extract (CFE150).

| Class | Family | Major activity | CON | CFE150[1] | Corrected *P*-value |
|---|---|---|---|---|---|
| Auxiliary activities | AA6 | 1,4-benzoquinone reductase | 1.04±0.07 | 1.15±0.06 | <0.01 |
| Carbohydrate Esterases | CE1 | Acetyl xylan esterase, feruloyl esterase | 2.94±0.08 | 3.12±0.06 | <0.01 |
| | CE10 | Arylesterase, carboxyl esterase | 2.43±0.07 | 2.53±0.06 | <0.01 |
| | CE2 | Acetyl xylan esterase | 0.52±0.02 | 0.59±0.01 | <0.01 |
| | CE3 | Acetyl xylan esterase | 0.48±0.03 | 0.56±0.03 | <0.01 |
| | CE4 | Acetyl xylan esterase, acetyl xylan esterase | 0.60±0.03 | 0.69±0.03 | <0.01 |
| | CE7 | Acetyl xylan esterase, cephalosporin-C deacetylase | 0.77±0.02 | 0.81±0.01 | <0.01 |
| | CE8 | Pectin methylesterase | 0.62±0.03 | 0.52±0.02 | <0.01 |
| | CE9 | N-acetylglucosamine 6-phosphate deacetylase, | 0.53±0.05 | 0.63±0.01 | <0.01 |
| Glycoside Hydrolases | GH10 | Endo-1,4-beta-xylanase, endo-1,3-beta-xylanase | 0.52±0.04 | 0.47±0.02 | <0.01 |
| | GH105 | Unsaturated rhamnogalacturonyl hydrolase | 0.53±0.02 | 0.47±0.02 | <0.01 |
| | GH106 | Alpha-L-rhamnosidase | 0.87±0.04 | 0.80±0.02 | <0.01 |
| | GH109 | Alpha-N-acetylgalactosaminidase | 0.53±0.03 | 0.57±0.01 | <0.05 |
| | GH115 | Xylan alpha-1,2-glucuronidase | 0.71±0.04 | 0.59±0.03 | <0.01 |
| | GH13 | Alpha-amylase, pullulanase | 11.40±0.16 | 12.03±0.10 | <0.01 |
| | GH28 | Polygalacturonase, alpha-L-rhamnosidase | 1.08±0.03 | 0.96±0.05 | <0.01 |
| | GH29 | Alpha-L-fucosidase | 0.64±0.02 | 0.59±0.03 | <0.05 |
| | GH32 | Invertase, endo-inulinase | 0.81±0.02 | 0.92±0.03 | <0.01 |
| | GH36 | Alpha-galactosidase | 0.92±0.05 | 1.09±0.04 | <0.01 |
| | GH43 | Beta-xylosidase, Alpha-L-arabinofuranosidase | 3.09±0.09 | 2.76±0.09 | <0.01 |
| | GH5 | Endo-beta-1,4-glucanase / cellulase | 20.41±0.19 | 19.90±0.23 | <0.01 |
| | GH51 | Endoglucanase | 0.90±0.05 | 0.82±0.03 | <0.05 |
| | GH53 | Amylomaltase or 4-alpha-glucanotransferase | 0.54±0.02 | 0.51±0.01 | <0.05 |
| | GH77 | Alpha-L-rhamnosidase | 1.11±0.04 | 1.23±0.014 | <0.01 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
|  | GH78 | Alpha-L-rhamnosidase | 1.04±0.03 | 1.12±0.04 | <0.01 |
|  | GH94 | Cellobiose phosphorylase | 0.84±0.04 | 0.99±0.04 | <0.01 |
|  | GH95 | Alpha-L-fucosidase | 0.90±0.04 | 0.76±0.03 | <0.01 |
|  | GH97 | Glucoamylase | 0.84±0.04 | 1.48±0.07 | <0.01 |
| Glycosyl Transferases | GT28 | 1,2-diacylglycerol 3-beta-galactosyltransferase | 0.49±0.03 | 0.55±0.01 | <0.05 |
|  | GT35 | Glycogen or starch phosphorylase | 1.20±0.08 | 1.52±0.07 | <0.01 |
|  | GT41 | UDP-GlcNAc:peptide beta-N-acetylglucosaminyltransferase | 2.31±0.05 | 2.19±0.07 | <0.01 |
|  | GT5 | UDP-Glc: glycogen glucosyltransferase | 0.81±0.04 | 0.94±0.03 | <0.01 |
| Polysaccharide Lyases | PL1 | Pectate lyase | 0.67±0.03 | 0.58±0.02 | <0.01 |
|  | PL11 | Rhamnogalacturonan endolyase | 0.57±0.03 | 0.45±0.03 | <0.01 |

[1] CFE, citrus flavonoids extract; AA, Auxiliary activities; CE, Carbohydrate esterases; GH, Glycoside hydrolases; GT, Glycosyl transferases; PL, Polysaccharide lyases.