|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Item | Number of observations | Number of Outliers | Item | Number of observations | Number of Outliers |
| DMI, kg/d | 63 | 0 | Total-tract apparent digestibility |
| Starch intake, kg/d | 63 | 0 |  DM | 63 | 0 |
| Milk yield, kg/d | 63 | 0 |  OM | 63 | 0 |
| Milk yield/DMI, kg/kg | 63 | 0 |  CP | 63 | 4 |
| 4% FCM,4 kg/d | 63 | 0 |  NDF | 63 | 0 |
| 4% FCM/DMI, kg/kg | 63 | 0 |  Starch | 63 | 0 |
| ECM,4 kg/d | 63 | 0 |  Ether extract | 63 | 0 |
| ECM/DMI, kg/kg | 63 | 0 | N intake, g/d | 63 | 4 |
| Milk fat, % | 63 | 1 | Urinary parameters |  |  |
| Milk fat, kg/d | 63 | 1 |  Creatinine, m*M* | 63 | 0 |
| Milk true protein, % | 63 | 1 |  Volume, L/d | 62 | 0 |
| Milk true protein, kg/d | 63 | 1 |  Urea N, g/d | 62 | 0 |
| Milk lactose, % | 63 | 1 |  Total N, g/d | 62 | 2 |
| Milk lactose, kg/d | 63 | 1 |  Urea N, % of total N | 61 | 2 |
| Milk SCC, ×1,000 cells/mL | 63 | 1 |  Urea N, % of N intake | 62 | 3 |
| Milk N, % of N intake | 62 | 3 |  Total N, % of N intake | 61 | 2 |
| MUN, mg/dL | 63 | 1 | N balance |  |  |
| PUN, mg/dL | 63 | 2 |  N intake, g/d | 63 | 4 |
| BCS | 64 | 0 |  Milk N, g/d | 63 | 4 |
| BCS change, point/21 d | 63 | 0 |  Urinary N, g/d | 62 | 2 |
| BW, kg | 63 | 0 |  Fecal N, g/d | 63 | 4 |
| BW change, kg/d | 60 | 0 |  Retained N, g/d | 62 | 3 |

**Supplemental Table S1.** Numbers of observations used in the statistical analyses and outliers removed from the data sets per variable of interest in a study whereby lactating dairy cows were fed low MP diets with different starch concentrations supplemented or not with rumen-protected Met, Lys, and His

**Supplemental Table S1.** Numbers of observations used in the statistical analyses and outliers removed from the data sets per variable of interest in a study whereby lactating dairy cows were fed low MP diets with different starch concentrations supplemented or not with rumen-protected Met, Lys, and His (continued)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Item | Number of observations | Number of Outliers | Item | Number of observations | Number of Outliers |
| EAA, µ*M* |  |  | Gaseous emissions |  |  |
|  Arg | 63 | 0 |  CO2, kg/d | 57 | 0 |
|  His | 63 | 0 |  CH4, g/d | 58 | 0 |
|  Ile | 63 | 1 |  CH4, g/kg of DMI | 58 | 0 |
|  Leu | 63 | 1 |  CH4, g/kg of ECM | 58 | 0 |
|  Lys | 63 | 0 | Fractions,Mcal/d |  |  |
|  Met | 63 | 0 |  GE intake | 63 | 0 |
|  Phe | 63 | 0 |  DE intake | 63 | 0 |
|  Thr | 63 | 0 |  ME intake | 56 | 1 |
|  Trp | 63 | 0 | Components,Mcal/d |  |  |
|  Val | 63 | 1 |  Fecal energy | 63 | 0 |
|  Total | 63 | 1 |  Urinary energy | 61 | 1 |
| NEAA, µ*M* |  |  |  CH4 energy | 58 | 0 |
|  Ala | 63 | 0 |  Milk energy | 63 | 1 |
|  Asn | 63 | 0 | Efficiencies, % | 62 | 0 |
|  Asp | 63 | 0 |  ME/DE | 56 | 1 |
|  Cit | 63 | 0 |  Milk energy/ME | 56 | 1 |
|  Cys | 63 | 0 |  |  |  |
|  Gln | 63 | 0 |  |  |  |
|  Glu | 63 | 1 |  |  |  |
|  Gly | 63 | 0 |  |  |  |
|  Orn | 63 | 0 |  |  |  |
|  Pro | 63 | 0 |  |  |  |
|  Ser | 63 | 1 |  |  |  |
|  Taurine | 63 | 1 |  |  |  |
|  Try | 63 | 0 |  |  |  |
|  Total | 63 | 3 |  |  |  |
| Carnosine | 63 | 1 |  |  |  |

**Supplemental Table S2.** NRC (2001) evaluation of the basal diets1 and supply of digestible His, Met, and Lys from the basal diets and rumen-protected (RP)-His, RP-Met, and RP-Lys2

|  |  |
| --- | --- |
|  | Pre-experiment3 |
| Item, g/d | HS | RSPA |
| Digestible His requirement4 | 61 | 61 |
| Digestible His supply | 59  | 57 |
| Digestible His balance |  −2 | −4 |
| Digestible His from RP-His |  3 |  3 |
|  |  |  |
| Digestible Met requirement4 | 61 | 61 |
| Digestible Met supply | 52 | 50 |
| Digestible Met balance | −9 | −11 |
| Digestible Met from RP-Met |  7 |  7 |
|  |  |  |
| Digestible Lys requirement4 | 182 | 182 |
| Digestible Lys supply | 181 | 183 |
| Digestible Lys balance |  −1 |  1 |
| Digestible Lys from RP-Lys |  2 |  2 |

1HS = high starch diet, RSPA = reduced starch + palmitic acid-enriched supplement.

212 g/d of RP-Met (Smartamine® M; Adisseo USA Inc.), 9 g/d of RP-Lys (AjiPro®-L; Ajinomoto Health & Nutrition North America Inc.), and 15 g/d of RP-His (prototype supplement; Ajinomoto Co. Inc.); RP-Met, RP-Lys, and RP-His supplements contained 75% DL-Met with 80% bioavailability (Chirgwin et al., 2015), 40% Lys with 54% bioavailability (Giallongo et al., 2016), and 40% His with 49% bioavailability (according to the manufacturer).

3All values were estimated using the NRC (2001) model for a dairy cow averaging 120 DIM, weighing 700 kg of BW, consuming 26 kg/d of DM, and producing 42 kg/d of milk with 3.5% fat, 3.1% true protein, and 4.98% lactose; the nutrient and AA composition of feeds obtained from previous studies done in our laboratory were used in the NRC (2001) software except for the AA profile of grass hay for which the NRC (2001) default values were adopted.

4Requirements of digestible His, Met, and Lys were calculated as 2.2, 2.2, and 6.6% of MP requirements, respectively (Schwab et al., 2005).

**Supplemental Table S3**. Nitrogen balance in lactating dairy cows fed low MP diets with different starch concentrations supplemented or not with rumen-protected Met, Lys, and His (RP-MLH1)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Diet2 |  | *P*-value3 |
| Item | HS | HS+AA | RSPA | RSPA+AA | SEM | SC | MLH | SC × MLH |
| N intake, g/d | 742 | 751 | 773 | 775 | 17.5 | <0.001 | 0.34 | 0.52 |
| Milk N, g/d | 225 | 228 | 224 | 226 | 6.01 | 0.65 | 0.42 | 0.89 |
| Urinary N, g/d | 281 | 273 | 337 | 334 | 7.20 | <0.001 | 0.26 | 0.63 |
| Fecal N, g/d | 217 | 211 | 205 | 204 | 7.27 | 0.06 | 0.49 | 0.66 |
| Retained N,2 g/d |  19.5 |  40.6 |  4.89 |  10.8 | 8.06 |  0.001 | 0.04 | 0.23 |

1RP-MLH = 12 g/d of RP-Met (Smartamine® M; Adisseo USA Inc.), 9 g/d of RP-Lys (AjiPro®-L; Ajinomoto Health & Nutrition North America Inc.), and 15 g/d of RP-His (prototype supplement; Ajinomoto Co. Inc.).

2HS = high starch; HS+AA = HS + RP-MLH; RSPA = reduced starch + palmitic acid-enriched supplement; and RSPA+AA = reduced starch + palmitic acid-enriched supplement + RP-MLH; BergaFat F100 (Berg+Schmidt America LLC), which is a ruminally stable lipid supplement containing 80% palmitic acid was fed at 1.5% of the diet DM.

3SC = main effect of dietary starch concentration, MLH = main effect of RP-MLH supplementation, and SC × MLH = interaction between dietary starch concentration and RP-MLH supplementation.

4Retained N = N intake – (milk N + urinary N + fecal N).