**Milk production responses and rumen fermentation of dairy cows supplemented with summer brassicas**

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Animal

Supplementary Material S1

Equations for microbial N estimation

1) Alantoin (mmol/day) = Alantoin (mmol/L) X VU (L/day)

2) Uric acid (mmol/day) = Uric Acid (mmol/L) x VU (L/day),

Where VU: Volume of Urine (L/day)

3) VU (L/day) = CT (mg/day)/ cCreat (mg/L),

Where:

VU: Volume of Urine (L/day)

CT (mg/day): total daily excreted creatinine

cCreat (mg/L): creatinine concentration in urine

4) CT (mg/day) = LW (kg) x C (mg/kg),

Where:

LW (kg): liveweight of cows

C (mg/kg): constant creatinine excretion of 26 mg/kg LW

5) DPe (mmol/day) = (DPT (mmol/L) x (LW (kg) x Kct)/ 113.12)/CT (mmol/L)

Where:

DPe (mmol/day): daily excretion of purine derivatives

DPT (mmol/L): daily concentration of total purine derivatives

LW (kg): liveweight of cows

Kct: coefficient of daily creatinine excretion (mg/day) = 113 \* LW-0,25 (113,12= creatinine molecular weight).

CT (mmol/L): creatinine concentration in the urine

6) PA (mmol/day) = (DPe (mmol/day)- (0.385 \* LW0.75))/0.85

Where:

PA (mmol/day): daily purines absorption

DPe (mmol/day): daily excretion of purine derivatives

7) MN (g/day) = (PA (mmol/day) \* 70 mg/mmol/(0.83\*0.116\*1000)

Where

MN: microbial nitrogen

PA: daily purines absorption

Supplementary Material S2 “Codes of statistical models”

Carryover effect:

Proc Mixed;

Class square period trt seq cow carry;

Model y = seq period trt carry;

random cow(seq);

Run;

Replicated Latin square:

Proc Mixed;

Class cow square period trt seq ani carry;

Model y = period trt;

random square cow(square);

lsmeans trt/ pdiff adjust= tukey;

Run;

Repeated measures

proc mixed data = WORK.'Juan Keim SCFA data verano'n;

class cow square period trt hour;

model y = period trt hour trt\*hour / ddfm=KR;

random square cow(square);

repeated hour / subject=cow(square) type=CS;

LSMEANS trt / pdiff adjust=tukey;

LSMEANS hour /pdiff adjust=tukey;

LSMEANS trt\*hour / pdiff ADJUST=TUKEY slice=hour;

run;

Supplementary Material S3

Results of quality control:

1.- Milk composition

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Measuring range | Performance range | Repeatability | Accuracy Single cow |
| Fat | 0-15% | 2-15% | CV < 0.5% | CV < 1.5% |
| Protein | 0-10% | 2-10% | CV < 0.5% | CV < 1.5% |
| Lactose | 0-10% | 2-10% | CV < 0.5% | CV < 1.5% |
| Solids | 0-20% | 2-20% | CV < 0.5% | CV < 1.5% |
| Urea patented | 10-100 mg/dl | 10-100 mg/dl | Sd < 1.5 mg/dl | Sd < 3.5 mg/dl |
| Somatic cell count (SCC) | 0 – 10 mill cells/ml | 0.1 – 1.5 mill | CV < 6% 100-299k SCC/mlCV < 4% 300-499k SCC/mlCV < 3% 500-1500k SCC/ml | < 10% relative mean diff. from DMSCC(Direct Microscopic Somatic Cell Count) |

CV = coefficient of variation; Sd = standard deviation

2.- pH sensors:

Temperature 18 – 25 C

EO (reference potential) Point +/- 25 mV

Stir Noise (pH6.86) +/- .1mV

Slope (pH4 – pH6.86) 161 – 174 mV

Drift (pH4) +/- 1000 uV

Na Slope > 440 mV

Junction Resistance 300 – 3200 ohms

Internal validation of pH bolus compared with rumen pH from cannulated cows

Average difference: 0.041

Standard deviation of difference: 0.137

Min difference: 0.02

Max difference: 0.26

Calculated Chi square: 0.406

Tabulated Chi square: 67.5

Calculated Chi square < Tabulated Chi square, no differences of rumen pH obtained from cannulas and bolus.

 3.- Purine derivatives:

Calibrations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | range | intercept | slope | r2 |
| Allantoin | 1 – 15 mmol/l | 2511 | 10834 | 0.996 |
| Uric Acid | 0 – 1 mmol/l | -2591 | 58535 | 0.999 |
| Creatinine | 1 – 5 mmol/l | -1522 | 67786 | 0.998 |

 4.- Volatile fatty acids and ammonia

Calibrations

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | range | intercept | slope | CV | r2 |
| Ammonia | 0 – 20 mM | 0.075 | 0.012 | 0.026 | 0.992 |
| Acetic | 10 – 100 mM | 86235 | 178747 | 0.001 | 0.999 |
| Propionic | 5 – 45 mM | -2101 | 449550 | 0.002 | 1.000 |
| Isobtyric | 0.1 – 5 mM | 1199 | 607354 | 0.002 | 0.993 |
| Butyric | 1 – 20 mM | 3861 | 654807 | 0.004 | 1.000 |
| Isovaleric | 0.1 – 5mM | -3347 | 818373 | 0.011 | 0.999 |
| Valeric | 0.1 – 5 mM | -2104 | 798597 | 0.017 | 0.999 |
| Caproic | 0.1 – 5 mM | 13603 | 922825 | 0.026 | 1.000 |