



Supplementary File

Table 2. Evaluation of feed rationing.

| Parameters Ration Guidelines | | | | Farms | | | | | | | | | | | | | |
|------------------------------|-------------------------|--------|--------|---------------|-------|--------|-------|--------|--------|--------|--------|--------|--------|-------|---------------|---------------|---------------|
| | | Lower | Upper | Target | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Means | Means Group A | Means Group B |
| Dry matter, kg/d | | | | | 20.16 | 20.48 | 20.96 | 20.64 | 21.12 | 20.64 | 21.06 | 20.48 | 20.64 | 20.64 | 20.68 | 20.70 | 20.70 |
| RDP % DM | | 11.50 | 12.00 | 11.00 | 9.50 | 12.00 | 9.00 | 14.00 | 13.80 | 13.20 | 9.30 | 10.4 | 9.60 | 9.30 | 11.01 | 13.30 | 9.70 |
| Soluble protein, % of CP | | 30.00 | 35.00 | 31.00 | 22.00 | 31.00 | 23.00 | 32.00 | 31.00 | 33.00 | 28.30 | 31.00 | 33.00 | 24.50 | 28.88 | 31.80 | 27.00 |
| Ruminal nitrogen balance | NH ₃ % needs | 105.00 | 150.00 | 110.00 | 98.00 | 112.00 | 92.00 | 130.00 | 128.00 | 132.00 | 98.50 | 106.00 | 108.00 | 98.50 | 110.00 | 124.00 | 101.80 |
| | Peptides % needs | 105.00 | 150.00 | 110.00 | 84.00 | 111.00 | 87.00 | 115.00 | 109.00 | 107.00 | 101.00 | 106.00 | 101.00 | 86.00 | 100.70 | 110.50 | 94.20 |
| Need of proteins Np/Mp % | | 60.00 | 70.00 | 65.00 | 58.00 | 63.00 | 58.00 | 63.00 | 64.00 | 68.00 | 57.00 | 63.00 | 68.00 | 57.00 | 61.90 | 64.50 | 60.20 |
| NDF%ss | | 28.00 | 35.00 | 30.00 | 33.40 | 34.80 | 31.00 | 36.40 | 34.80 | 35.60 | 35.30 | 36.20 | 35.40 | 34.80 | 34.77 | 31.60 | 28.60 |
| NDF forage/NDF tot % | | 65.00 | 75.00 | 75.00 | 60.00 | 68.00 | 72.00 | 68.00 | 72.00 | 71.00 | 69.50 | 68.00 | 71.00 | 69.50 | 68.90 | 69.80 | 68.30 |
| peNDF % DM | | 21.00 | 28.00 | 23.00 | 20.69 | 27.52 | 21.62 | 29.76 | 29.96 | 28.98 | 16.67 | 30.40 | 25.32 | 20.58 | 25.15 | 29.10 | 22.50 |
| NDF fermentable % DM | | 9.00 | 12.00 | 10.00 | 16.00 | 22.00 | 15.60 | 22.00 | 18.00 | 20.00 | 15.80 | 22.00 | 20.00 | 15.8 | 18.72 | 20.50 | 17.50 |
| Starch | | 21.00 | 27.00 | 24.00 | 28.30 | 24.00 | 27.40 | 23.80 | 24.00 | 25.00 | 29.20 | 28.76 | 28.30 | 29.00 | 26.78 | 24.20 | 28.50 |
| Fermentable starch % DM | | 20.00 | 22.00 | 21.00 | 21.75 | 17.60 | 22.50 | 15.20 | 14.30 | 15.80 | 21.00 | 17.60 | 17.00 | 22.00 | 18.48 | 15.70 | 20.30 |
| Soluble fiber % DM | | 5.00 | 11.00 | 7.00 | 5.11 | 7.00 | 6.00 | 7.00 | 6.80 | 7.00 | 5.00 | 7.00 | 7.00 | 5.00 | 6.29 | 7.00 | 5.90 |
| NFC % DM | | 35.00 | 39.00 | 38.00 | 42.00 | 40.00 | 43.00 | 40.00 | 38.70 | 39.50 | 41.00 | 40.00 | 39.50 | 41.00 | 40.47 | 39.60 | 41.10 |
| Lys% Mp | | 6.50 | 7.30 | 6.57 | 4.92 | 6.50 | 5.70 | 6.50 | 6.50 | 6.70 | 4.90 | 6.50 | 6.70 | 4.90 | 5.98 | 6.60 | 5.60 |
| Met% Mp | | 2.10 | 2.50 | 2.12 | 1.85 | 2.10 | 1.96 | 2.10 | 2.10 | 2.30 | 1.80 | 2.10 | 2.30 | 1.80 | 2.04 | 2.20 | 2.00 |
| Lys/Met | | 3.00 | 3.30 | 3.10 | 2.66 | 2.70 | 2.75 | 2.82 | 2.74 | 2.89 | 2.56 | 2.70 | 2.89 | 2.56 | 2.73 | 2.80 | 2.70 |

DM, dry matter; RDP, rumen degradable protein; Np/Mp, net protein/ metabolizable protein; NDF, neutral detergent fiber; peNDF, physically effective NDF; NFC, non-fibrous carbohydrates; Lys, lysine; Met, methionine.

Table 3. Unifeed quality assessment with Penn State particle separator.

| Sieve plates | Guidelines values | | Farms | | | | | | | | | | Means | | Means | Means |
|----------------|-------------------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|-------|-------|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Group A | Group B | | |
| Upper (%) | 2 | 8 | 9.30 | 10.00 | 7.80 | 8.00 | 6.00 | 7.00 | 5.00 | 8.00 | 10.00 | 7.00 | 7.81 | 7.75 | 7.85 | |
| Middle (%) | 30 | 50 | 27.30 | 27.00 | 25.20 | 34.00 | 34.60 | 40.00 | 35.00 | 33.00 | 31.00 | 31.00 | 31.81 | 33.90 | 30.42 | |
| Lower (%) | 30 | 50 | 48.00 | 49.00 | 51.00 | 51.00 | 53.00 | 48.00 | 42.00 | 44.00 | 42.00 | 42.00 | 47.00 | 50.25 | 44.83 | |
| Bottom pan (%) | 5 | 20 | 15.40 | 14.00 | 16.00 | 7.00 | 6.40 | 5.00 | 18.00 | 15.00 | 17.00 | 20.00 | 13.38 | 8.10 | 16.90 | |
| | | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |

Table 4. Quantitative and qualitative parameters of the productions.

| Farms | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Means | Means | Means |
|-----------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| | | | | | | | | | | | | Group A | Group B |
| Means for farm (kg of milk) | 30.60 | 27.00 | 28.00 | 31.00 | 25.00 | 29.60 | 26.00 | 24.00 | 25.00 | 28.00 | 27.42 | 28.15 | 26.93 |
| Fat % | 3.60 | 3.93 | 3.50 | 3.91 | 4.10 | 3.75 | 3.84 | 3.98 | 3.92 | 3.42 | 3.80 | 3.92 | 3.71 |
| Proteins % | 3.24 | 3.54 | 3.15 | 3.52 | 3.69 | 3.38 | 3.46 | 3.58 | 3.53 | 3.25 | 3.43 | 3.53 | 3.37 |
| Lactose % | 4.90 | 4.80 | 4.70 | 4.80 | 4.90 | 4.80 | 4.90 | 4.90 | 4.80 | 4.80 | 4.83 | 4.83 | 4.83 |
| Urea in milk (mg/100 mL) | 21.00 | 29.00 | 22.00 | 30.10 | 30.30 | 31.00 | 21.98 | 21.70 | 22.48 | 21.00 | 25.06 | 30.10 | 21.69 |
| Somatic cells (n/mL) | 210000 | 190000 | 180000 | 190000 | 200000 | 195000 | 165000 | 178000 | 165000 | 145000 | 181800 | 193750 | 173833 |
| Microbial load (TBC/mL) | 10000 | 50000 | 80000 | 90000 | 120000 | 98000 | 88000 | 45000 | 38000 | 27000 | 64600 | 89500 | 48000 |

TBC, total bacterial count.

Table 5. Management parameters.

| Farms | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Means | Means | Means |
|--|-------|------|-------|------|------|------|------|------|-------|-------|-------|---------|---------|
| | | | | | | | | | | | | Group A | Group B |
| Body condition score (BCS) mean | 3.75 | 3.25 | 3.50 | 3.50 | 3.00 | 3.25 | 3.00 | 3.25 | 3.00 | 3.25 | 3.28 | 3.25 | 3.29 |
| Lameness (laminitis) % (of total lactating cows) | 15.00 | 7.00 | 13.00 | 7.20 | 8.50 | 4.50 | 6.40 | 5.40 | 10.30 | 15.60 | 9.29 | 6.80 | 10.95 |

Table 6. Fecal consistency.

| Frequency of class % | | | | | | | |
|----------------------|---------------------|-------|-------|-------|-------|-------|-----------------|
| Farms | Physiological stage | 1FC | 2FC | 3FC | 4FC | 5FC | N. Observations |
| 1 | Lactation | 9.52 | 33.33 | 57.14 | 0.00 | 0.00 | 21 |
| 2 | Lactation | 11.11 | 37.04 | 18.52 | 18.52 | 14.81 | 27 |
| 3 | Lactation | 27.78 | 11.11 | 33.33 | 27.78 | 0.00 | 18 |
| 4 | Lactation | 33.33 | 11.11 | 55.56 | 0.00 | 0.00 | 18 |
| 5 | Lactation | 30.77 | 38.46 | 30.77 | 0.00 | 0.00 | 13 |
| 6 | Lactation | 26.67 | 20.00 | 53.33 | 0.00 | 0.00 | 15 |
| 7 | Lactation | 46.15 | 30.77 | 23.08 | 0.00 | 0.00 | 13 |
| 8 | Lactation | 18.18 | 54.55 | 27.27 | 0.00 | 0.00 | 11 |
| 9 | Lactation | 35.71 | 28.57 | 14.29 | 21.43 | 0.00 | 14 |
| 10 | Lactation | 27.27 | 18.18 | 54.55 | 0.00 | 0.00 | 11 |
| Means | | 26.65 | 28.31 | 36.78 | 6.77 | 1.48 | |
| Means Group A | | 25.47 | 26.65 | 39.55 | 4.63 | 3.70 | |
| Means Group B | | 27.44 | 29.42 | 34.94 | 8.20 | 0.00 | |

1FC, runny: liquid consistency, splatters on impact, spreads readily (not optimal); 2FC, loose: may pile slightly and spreads and splatters moderately on impact and setting (not optimal); 3FC, soft: firm but not hard, piles but spreads slightly on impact and settling (optimal); 4FC, soft, the fall have a dull sound, well circumscribed and are layered circles distinguishable (not optimal); 5FC, dry, hard, dry appearance, original form not distorted on impact and settling (not optimal).

Table 7. Undigested Fraction analysis.

| Frequency of class % | | | | | | | |
|----------------------|---------------------|-------|-------|-------|-------|------|-----------------|
| Farms | Physiological stage | 1UF | 2UF | 3UF | 4UF | 5UF | N. Observations |
| 1 | Lactation | 6.67 | 46.67 | 6.67 | 40.00 | 0.00 | 21 |
| 2 | Lactation | 62.00 | 25.00 | 11.00 | 2.00 | 0.00 | 12 |
| 3 | Lactation | 29.41 | 47.06 | 5.88 | 17.65 | 0.00 | 17 |
| 4 | Lactation | 75.00 | 13.00 | 12.00 | 0.00 | 0.00 | 18 |
| 5 | Lactation | 68.00 | 14.00 | 13.00 | 5.00 | 0.00 | 15 |
| 6 | Lactation | 74.00 | 14.00 | 12.00 | 0.00 | 0.00 | 22 |
| 7 | Lactation | 20.00 | 50.00 | 30.00 | 0.00 | 0.00 | 20 |
| 8 | Lactation | 50.00 | 25.00 | 25.00 | 0.00 | 0.00 | 8 |
| 9 | Lactation | 14.29 | 35.71 | 28.57 | 21.43 | 0.00 | 14 |
| 10 | Lactation | 30.77 | 46.15 | 23.08 | 0.00 | 0.00 | 13 |
| Means | | 43.01 | 31.66 | 16.72 | 8.61 | 0.00 | |
| Means Group A | | 69.75 | 16.50 | 12.00 | 1.75 | 0.00 | |
| Means Group B | | 25.19 | 41.77 | 19.87 | 13.18 | 0.00 | |

1UF, normal feces with undigested food particles less than 1.3 mm; 2UF, unusual feces particles with long forage; 3UF, unusual feces, presence of cotton seeds intact; 4UF, unusual feces, presence of concentrates; 5UF, unusual feces, presence of mucin and gases.

Table 8. Reproductive parameters.

| Farms | DO (die) | CI (die) | Assp (n.) | Cr (%) | Sr (%) | Pr (%) |
|----------------------|---------------|---------------|-------------|---------------|---------------|---------------|
| 1 | 103 | 385 | 1.16 | 56.00% | 30.00% | 17.00% |
| 2 | 166 | 448 | 2.50 | 40.00% | 36.00% | 15.00% |
| 3 | 111 | 393 | 1.25 | 39.00% | 32.00% | 12.00% |
| 4 | 153 | 435 | 2.55 | 42.00% | 41.00% | 17.00% |
| 5 | 156 | 438 | 2.67 | 38.00% | 49.00% | 18.00% |
| 6 | 163 | 445 | 2.63 | 38.00% | 39.00% | 15.00% |
| 7 | 121 | 403 | 1.56 | 50.00% | 33.00% | 16.00% |
| 8 | 145 | 427 | 2.22 | 86.00% | 38.00% | 32.00% |
| 9 | 143 | 425 | 2.00 | 41.00% | 34.00% | 14.00% |
| 10 | 107 | 389 | 1.21 | 48.00% | 39.00% | 19.00% |
| Means | 136.80 | 418.80 | 1.98 | 47.80% | 37.10% | 17.50% |
| Means Group A | 159.50 | 441.50 | 2.59 | 39.50% | 41.25% | 16.25% |
| Means Group B | 121.67 | 403.67 | 1.57 | 53.33% | 34.33% | 18.33% |

DO, day open; CI, Calvin interval; Assp, number of inseminations for pregnancy; Cr, conception rate; Sr, service rate; Pr, pregnancy rate.

Table 9. Farms income parameters.

| Farms | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Means | Means Group A | Means Group B |
|--|------|------|------|------|------|------|------|------|------|------|-------------|---------------|---------------|
| Income over feed cost (€) | 6.36 | 5.61 | 6.14 | 7.33 | 4.80 | 6.53 | 5.00 | 4.37 | 4.25 | 5.81 | 5.62 | 6.07 | 5.32 |
| Milk margin (€) | 0.21 | 0.21 | 0.22 | 0.24 | 0.19 | 0.22 | 0.19 | 0.18 | 0.17 | 0.21 | 0.20 | 0.22 | 0.20 |
| Feed cost per kilogram of dry matter (€/kg) | 0.34 | 0.29 | 0.28 | 0.29 | 0.28 | 0.30 | 0.29 | 0.29 | 0.31 | 0.30 | 0.30 | 0.29 | 0.30 |
| Milk price (€/kg) | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 |
| Feed efficiency (kg of milk/kg dry matter) | 1.54 | 1.41 | 1.34 | 1.60 | 1.30 | 1.49 | 1.30 | 1.26 | 1.29 | 1.34 | 1.39 | 1.45 | 1.35 |

Table 10. Correlations between urea content, days open (DO), Calving interval (CI), number of inseminations for pregnancy (Assp), conception rate (Cr), service rate (Sr), undigested fraction (UF), pregnancy rate (PR), fat content, rumen degradable protein (RDP).

| | Urea | DO | CI | Assp | Cr | Sr | 2UF | Fat |
|-------------|----------------------|----------|----------|----------|----------|--------|----------|--------|
| Urea | - | | | | | | | |
| DO | 0.928*** | - | | | | | | |
| CI | 0.928*** | 1 | - | | | | | |
| Assp | 0.961*** | 0.98** | 0.98*** | - | | | | |
| Cr | n.d. | n.d. | n.d. | n.d. | - | | | |
| SR | -0.691* | n.d. | n.d. | 0.69* | n.d. | - | | |
| PR | -0.037 ^{NS} | n.d. | n.d. | n.d. | 0.905*** | n.d. | - | |
| Fat | 0.669* | 0.778* | 0.778** | 0.806** | n.d. | n.d. | -0.635* | - |
| RDP | 0.912*** | 0.896*** | 0.896*** | 0.951*** | n.d. | 0.725* | -0.815** | 0.759* |

*P<0.05; **P<0.01; ***P<0.001; n.d., not detected.

Table 11. Correlation between urea, rumen degradable proteins (RDP) and peptides contents.

| | Urea | RDP | Peptides |
|-----------------------|----------|----------|----------|
| Urea | - | | |
| RDP | 0.912*** | - | |
| Peptides | 0.884*** | 0.868*** | - |
| NH₃ | 0.861*** | 0.934*** | 0.744*** |

*P<0.05; ** P<0.01; ***P<0.001.

Table 12. Correlations between physically effective neutral detergent fiber (peNDF), starch content, lameness, fecal consistency (FC), undigested fraction (UF), particle distribution plates of sieve.

| | peNDF | Starch | Lamness | 1FC | 5FC | 2UF | 4UF | Upper | Middle |
|---------|---------|-----------|----------|--------|----------|----------|---------|---------|----------|
| peNDF | - | - | - | - | - | - | - | - | - |
| Starch | - | - | - | - | - | - | - | - | - |
| Lamness | -0.635* | 0.837** | - | - | - | - | - | - | - |
| 1FC | - | - | - | - | - | - | - | - | - |
| 2FC | - | - | - | - | - | - | - | - | - |
| 3FC | - | - | - | - | - | - | - | - | - |
| 4FC | - | - | - | - | - | - | - | - | - |
| 5FC | - | - | - | - | - | - | - | - | - |
| 1UF | - | - | - | - | - | - | - | - | - |
| 2UF | - | 0.823** | - | - | - | - | - | - | - |
| 3UF | 0.644* | - | - | 0.703* | - | - | - | - | - |
| 4UF | - | - | - | - | - | - | - | - | - |
| 5UF | - | - | - | - | 1.000*** | - | - | - | - |
| Upper | - | - | - | - | - | - | 0.769** | - | - |
| Middle | 0.768** | - | - | - | - | - | -0.737* | -0.664* | - |
| Lower | - | - | - | - | - | - | - | - | - |
| Bottom | - | 0.704* | - | - | - | - | - | - | -0.833** |
| Fat | 0.701* | -0.920*** | -0.773** | - | - | -0.635* | - | - | - |
| RDP | - | -0.851** | - | - | - | -0.815** | - | - | - |

*P≤0.05; **P≤0.01; ***P≤0.001.

1UF, normal feces with undigested food particles less than 1.3 mm; 2UF, unusual feces particles with long forage; 3UF, unusual feces, presence of cotton seeds intact; 4UF, unusual feces, presence of concentrates; 5UF, unusual feces, presence of mucin and gases.

1FC, runny: liquid consistency, splatters on impact, spreads readily (not optimal); 2FC, loose: may pile slightly and spreads and splatters moderately on impact and setting (not optimal); 3FC, soft: firm but not hard, piles but spreads slightly on impact and settling (optimal); 4FC, soft, the fall have a dull sound, well circumscribed and are layered circles distinguishable (not optimal); 5FC, dry, hard, dry appearance, original form not distorted on impact and settling (not optimal). RDP, rumen degradable proteins.

Table 13. Correlations between income over feed cost (IOFC), milk margin (MM), feed efficiency (FE) and fat corrected milk (FCM).

| | Income over feed cost | Milk margin (MM) | Feed efficiency | FCM |
|-----------------------|-----------------------|------------------|-----------------|--------|
| Income over feed cost | - | - | - | - |
| Milk margin (MM) | 0.967*** | - | - | - |
| Feed efficiency | 0.895*** | 0.789** | - | - |
| FCM | 0.912*** | 0.824** | 0.989*** | - |
| 3FC | 0.793** | 0.707* | 0.723** | 0.711* |
| 2FC | -0.709** | -0.693* | - | - |

*P≤0.05; **P≤0.01; ***P≤0.001.

2FC, loose: may pile slightly and spreads and splatters moderately on impact and setting (not optimal); 3FC, soft: firm but not hard, piles but spreads slightly on impact and settling (optimal).