

# Using milk components to estimate the risk of energy imbalance in Holstein cows by means of receiver operating characteristic (ROC) analysis

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## Electronic Supplementary Material (ESM)

**Table S1.** Least square means (LSM) and standard errors (SE) for serum NEFA, afternoon milk yield, milk composition, and fatty acid composition recorded in participating farms from 5 to 35 days in milk

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Table S1. Least square means (LSM) and standard errors (SE) for serum NEFA, afternoon milk yield, milk composition, and fatty acid composition recorded in participating farms from 5 to 35 days in milk

| Variable                          | Farm 1<br><i>n</i> = 80   | Farm 2<br><i>n</i> = 192 | Farm 3<br><i>n</i> = 165  | Farm 4<br><i>n</i> = 46   | Farm 5<br><i>n</i> = 209  | <i>P</i> -value |
|-----------------------------------|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|-----------------|
|                                   | LSM ± SE                  | LSM ± SE                 | LSM ± SE                  | LSM ± SE                  | LSM ± SE                  |                 |
| NEFA (mmol/l)                     | 0.45 ± 0.05               | 0.32 ± 0.02              | 0.33 ± 0.03               | 0.39 ± 0.04               | 0.36 ± 0.02               | 0.056           |
| Milk yield (kg/afternoon milking) | 13.3 ± 0.60 <sup>c</sup>  | 18.2 ± 0.24 <sup>b</sup> | 19.2 ± 0.35 <sup>a</sup>  | 19.0 ± 0.57 <sup>a</sup>  | 17.5 ± 0.25 <sup>b</sup>  | <0.001          |
| Fat (%)                           | 4.2 ± 0.13 <sup>a</sup>   | 3.7 ± 0.05 <sup>b</sup>  | 4.4 ± 0.08 <sup>a</sup>   | 4.2 ± 0.12 <sup>a</sup>   | 4.4 ± 0.05 <sup>a</sup>   | <0.001          |
| Protein (%)                       | 3.3 ± 0.05                | 3.3 ± 0.02               | 3.4 ± 0.03                | 3.4 ± 0.05                | 3.3 ± 0.02                | 0.418           |
| FPR                               | 1.22 ± 0.03 <sup>b</sup>  | 1.12 ± 0.02 <sup>c</sup> | 1.34 ± 0.02 <sup>a</sup>  | 1.28 ± 0.03 <sup>ab</sup> | 1.32 ± 0.02 <sup>a</sup>  | <0.001          |
| Lactose (%)                       | 5.01 ± 0.03 <sup>a</sup>  | 5.01 ± 0.01 <sup>a</sup> | 4.95 ± 0.02 <sup>ab</sup> | 4.91 ± 0.03 <sup>b</sup>  | 4.98 ± 0.01 <sup>a</sup>  | <0.001          |
| MUN (mg/100 ml)                   | 20.9 ± 1.02 <sup>b</sup>  | 23.7 ± 0.41 <sup>a</sup> | 23.6 ± 0.59 <sup>a</sup>  | 18.5 ± 0.96 <sup>c</sup>  | 22.8 ± 0.42 <sup>ab</sup> | <0.011          |
| Citrate (%)                       | 0.14 ± 0.01 <sup>b</sup>  | 0.13 ± 0.00 <sup>c</sup> | 0.13 ± 0.00 <sup>c</sup>  | 0.10 ± 0.01 <sup>d</sup>  | 0.16 ± 0.00 <sup>a</sup>  | <0.001          |
| C14:0 (g/100 g fat)               | 9.08 ± 0.24 <sup>b</sup>  | 9.15 ± 0.09 <sup>b</sup> | 9.83 ± 0.14 <sup>a</sup>  | 7.72 ± 0.22 <sup>c</sup>  | 7.97 ± 0.10 <sup>c</sup>  | <0.001          |
| C16:0 (g/100 g fat)               | 24.9 ± 0.58 <sup>b</sup>  | 26.0 ± 0.23 <sup>b</sup> | 27.9 ± 0.33 <sup>a</sup>  | 25.9 ± 0.53 <sup>b</sup>  | 22.1 ± 0.23 <sup>c</sup>  | <0.001          |
| C18:0 (g/100 g fat)               | 11.1 ± 0.28 <sup>bc</sup> | 10.6 ± 0.11 <sup>c</sup> | 11.2 ± 0.16 <sup>b</sup>  | 10.6 ± 0.26 <sup>c</sup>  | 14.5 ± 0.11 <sup>a</sup>  | <0.001          |
| C18:1 (g/100 g fat)               | 27.4 ± 0.72 <sup>b</sup>  | 28.8 ± 0.28 <sup>b</sup> | 28.2 ± 0.42 <sup>b</sup>  | 38.5 ± 0.66 <sup>a</sup>  | 28.6 ± 0.29 <sup>b</sup>  | <0.001          |
| SCFA (g/100 g fat)                | 11.0 ± 0.28 <sup>c</sup>  | 11.3 ± 0.11 <sup>c</sup> | 12.2 ± 0.16 <sup>b</sup>  | 13.8 ± 0.27 <sup>a</sup>  | 10.2 ± 0.12 <sup>d</sup>  | <0.001          |
| MCFA (g/100 g fat)                | 34.7 ± 0.97 <sup>c</sup>  | 37.0 ± 0.38 <sup>b</sup> | 38.3 ± 0.56 <sup>a</sup>  | 36.1 ± 0.89 <sup>bc</sup> | 30.1 ± 0.39 <sup>d</sup>  | <0.001          |
| LCFA (g/100 g fat)                | 37.1 ± 0.61 <sup>c</sup>  | 37.7 ± 0.36 <sup>c</sup> | 39.0 ± 0.40 <sup>b</sup>  | 43.2 ± 0.74 <sup>a</sup>  | 42.5 ± 0.35 <sup>a</sup>  | <0.001          |
| SFA (g/100 g fat)                 | 60.8 ± 0.78 <sup>b</sup>  | 61.4 ± 0.31 <sup>b</sup> | 65.0 ± 0.45 <sup>a</sup>  | 66.3 ± 0.72 <sup>a</sup>  | 57.5 ± 0.32 <sup>c</sup>  | <0.001          |
| MUFA (g/100 g fat)                | 29.4 ± 0.69 <sup>c</sup>  | 29.6 ± 0.27 <sup>c</sup> | 29.4 ± 0.40 <sup>c</sup>  | 35.7 ± 0.64 <sup>a</sup>  | 30.8 ± 0.28 <sup>b</sup>  | <0.001          |
| PUFA (g/100 g fat)                | 2.61 ± 0.11 <sup>c</sup>  | 3.02 ± 0.04 <sup>b</sup> | 3.13 ± 0.06 <sup>b</sup>  | 2.70 ± 0.10 <sup>c</sup>  | 3.29 ± 0.05 <sup>a</sup>  | <0.001          |
| tUFA (g/100 g fat)                | 34.2 ± 0.78 <sup>b</sup>  | 33.7 ± 0.31 <sup>b</sup> | 30.0 ± 0.45 <sup>c</sup>  | 28.7 ± 0.72 <sup>c</sup>  | 37.5 ± 0.32 <sup>a</sup>  | <0.001          |

<sup>a-d</sup>Values with different manuscripts differ at *P* < 0.05

NEFA = non-esterified fatty acids; FPR = fat to protein ratio; LCFA = long-chain fatty acids; MCFA = medium-chain fatty acids; MUFA = monounsaturated fatty acids; MUN = milk urea nitrogen; PUFA = polyunsaturated fatty acids; SCFA = short-chain fatty acids; SFA = saturated fatty acids; tUFA = total unsaturated fatty acids