**Milk yield and composition responses to change in predicted net energy and metabolizable protein: A meta-analysis**

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**Supplementary file – for Online Publication Only**

Supplementary Material S1. The list of references used for the meta-analysis

Supplementary Material S2. INRA Systali feed units system

Supplementary Table S1. Random model outputs with dietary NEL and MP contents as co-variables

Supplementary Table S2. Random model outputs with NEL100 and MP67 supply as co-variables

Supplementary Table S3. Effect of weighting the observations on model coefficients estimate: Milk yield response to change in NEL and MP contents as an example

Supplementary Table S4. Main ingredient of the investigated treatments

**Supplementary Material S1. The list of references used for the meta-analysis**

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**Supplementary Material S2. INRA Systali feed units system**

*Brief description of recent updates*

Full details for the calculations of net energy for lactation (NEL) and metabolizable protein (MP) values are given in Sauvant and Nozière, 20161. Briefly the update of INRA Systali feed units system consisted on quantifying the effect of digestive interactions on nutrients supplies and subsequently on NEL and MP values by numerous meta-analyses of literature data. The main causes of digestive interactions included in the model are the feed intake level (DMI, % BW), the dietary proportion of concentrate and the rumen protein balance (RPB). The latter which is the difference between CP intake and CP flow to the duodenum (NH3 excluded) is also used, together with the fermentable organic matter to calculate the amount of microbial CP measured at duodenum. Therefore, the former distinction between MP limited by energy (PDIE) and MP limited by N (PDIN) used in the previous version of the INRA MP system is no longer applied and replaced by the couple, MP and RPB (Sauvant and Nozière, 2016)1. Practically, MP values are equivalent to the PDIE and are expressed in grams of protein digested in the small intestine supplied by rumen undegradable protein and by microbial protein.

*Calculation of non-productive N requirements*

The MP above maintenance was calculated according to the recent update of INRA protein unit system (Sauvant *et al.*, 2015)2. In this paper, three major losses of N, resulting in non-productive N requirements, were quantified. In order of importance they are the faecal metabolic CP losses, calculated from DMI (kg/d) and organic matter non-digested (OMnd, g/kg DM) as = DMI \* [5 \* (0.57 + 0.0074 \* OMnd)] / MP efficiency, the urinary endogenous N losses = 0.312 \* BW, and the scurf CP losses = (0.2 \* BW0.6) / MP efficiency. It should be noted that the same MP efficiency, conversion of MP above maintenance into milk protein, is also used for scurf CP losses, for faecal metabolic CP losses and for body protein retention/mobilisation (g/d). This last item is indexed on energy balance (EB) (= 4.479 \* EB / MP efficiency). The MP efficiency is calculated iteratively by using an initial value of 0.67, value used as reference in the NRC protein unit system (2001)3.

*Calculation of energy balance*

For energy, the last updates of the French unit system were applied (Sauvant *et al.*, 2015)4. NEL requirement for milk expressed in MJ per day was calculated from milk fat content (MFC, g/kg) and milk protein content (MPC, g/kg), according to Faverdin *et al.* (2007)5, as: 7.1 x (kg of milk x (0.44 + (0.0055 x (MFC – 40)) + (0.0033 x (MPC – 31)))) where the factor 7.1 converts the French energy unit UFL into MJ.

In the current work, EB was expressed as NEL in MJ per day and calculated as: [daily DMI x dietary metabolizable energy (ME) content – (0.607 x BW0.75 + daily NEL requirement for milk / kls ] x kgt where: 0.607 represents the ME requirement for maintenance. The coefficient kls is the efficiency to convert dietary ME into milk NEL, calculated as kls = 0.65 + 0.247 x [(ME/GE) - 0.63] with GE as gross energy. The coefficient kgt is the efficiency to convert NEL from body reserves into milk NEL and also to convert dietary ME into NEL body reserves. The coefficient kgt is calculated as follow: kgt = kls + 0.15.

1Sauvant D and Nozière P 2016. The quantification of the main digestive processes in ruminants: the equations involved in the renewed energy and protein feed evaluation systems. Animal, Accepted.

2Sauvant D, Cantalapiedra-Hijar G, Delaby L, Daniel JB, Faverdin P and Nozière P 2015. Actualisation des besoins protéiques des ruminants et détermination des réponses des femelles laitières aux apports de protéines digestibles dans l’intestin (PDI). INRA Production Animales, 28: 347-368.

3National Research Council 2001. Nutrient requirements of dairy cattle, Seventh revised edition. National Academy Press, Washington DC.

4Sauvant D, Ortigues-Marty I, Giger-Reverdin S and Nozière P 2015. Actualisation des besoins et efficacités énergétiques des femelles laitières. Rencontre Recherche ruminants 2015, 22: 225-228.

5Faverdin P, Delagarde R, Delaby L and Meschy F 2007. Alimentation des vaches laitières. In: Alimentation des bovins, ovins et caprins. Besoins des animaux - Valeur des aliments - Tables INRA 2007, mise à jour 2010. pp. 23-58. Editions Quae, Versailles, France.

**Supplementary Table S1. Random model outputs with dietary NEL and MP contents as co-variables**

**Table S1** Responses of milk yield and milk composition to changes in dietary NEL content (MJ/kg DM) and MP content (g/kg DM). The co-variables are mean centred on: NEL = 6.7 MJ/kg DM, MP = 100 g/kg DM.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Nexp | Intercept | Linear NEL | Quadratic NEL | Linear MP | Quadratic MP | NEL x MP | Outlier (%) | RMSE |
| Milk (MJ/d) | 278 | 96.14 (1.07)1 | **\_\_** | -5.25 (0.96) | 0.294 (0.024) | -0.0088 (0.0010) | 0.185 (0.045) | 2.4 | 3.81 |
| Milk (kg/d) | 279 | 32.15 (0.39) | 0.96 (0.28) | -1.12 (0.34) | 0.110 (0.009) | -0.0030 (0.0003) | 0.050 (0.016) | 2.1 | 1.33 |
| Milk component yields (g/d) |
| Fat | 279 | 1166 (15) | -54.3 (11.5) | -70.5 (13.2) | 2.90 (0.37) | -0.078 (0.014) | **\_\_** | 2.1 | 57.3 |
| Protein | 280 | 1009 (11) | 53.0 (10.1) | -35.2 (12.4) | 3.83 (0.33) | -0.126 (0.013) | 2.64 (0.59) | 2.2 | 49.0 |
| Lactose | 177 | 1546 (24) | 49.1 (18.7) | -80.5 (22.7) | 6.32 (0.68) | -0.15 (0.03) | 2.94 (1.10) | 0.9 | 77.1 |
| Milk component contents (g/kg) |
| Fat | 280 | 36.71 (0.31) | -2.29 (0.31) | -0.96 (0.37) | -0.056 (0.010) | **\_\_** | **\_\_** | 1.8 | 1.63 |
| Protein | 276 | 31.59 (0.14) | 0.69 (0.13) | **\_\_** | 0.018 (0.004) | -0.0010 (0.0002) | 0.025 (0.007) | 2.3 | 0.63 |
| Lactose | 174 | 47.82 (0.15) | **\_\_** | -0.38 (0.10) | **\_\_** | **\_\_** | **\_\_** | 2.2 | 0.44 |

Nexp=Number of experimental groups; Outlier = Observations with studentized residuals higher than 3 (or lower than -3); RMSE=Root mean square error after adjusting for the effect of experiment.

1 Standard errors of the coefficient are reported in parentheses.

Models were chosen based on AIC (see material and methods). All coefficients were significantly different from 0 at least at the level P<0.05.

These coefficients can be used to predict milk responses within the ranges of 5.9 – 7.6 MJ/kg DM for NEL and 73 – 121 g/kg DM for MP (means ± 2 SD), which reflect the current dataset.

**Supplementary Table S2. Random model outputs with NEL100 and MP67 supplies as co-variables**

**Table S2** Responses of milk yield and milk composition to changes in NEL100 supply (MJ/d) and MP67 supply (kg/d). The co-variables are mean centred on supplies for which: NEL in milk/NEL supply above maintenance = 1.00, Milk protein yield/MP above maintenance = 0.67.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Nexp | Intercept | Linear NEL100 | Quadratic NEL100 | Linear MP67 | Quadratic MP67 | Outlier (%) | RMSE |
| Milk (MJ/d) | 277 | 95.0 (1.2)1 | 0.158 (0.017) | -0.0020 (0.0005) | 14.88 (1.14) | -17.34 (2.24) | 1.5 | 2.93 |
| Milk (kg/d) | 279 | 31.66 (0.42) | 0.077 (0.005) | -0.00034 (0.00014) | 5.30 (0.35) | -3.33(0.69) | 1.3 | 0.88 |
| Milk component yields (g/d) |
| Fat | 278 | 1157 (15) | \_\_ | -0.026 (0.008) | 174.6(15.9) | -240.8(38.9) | 2.2 | 50.8 |
| Protein | 277 | 997 (12) | 3.093 (0.180) | -0.023 (0.005) | 184.5(12.4) | -192.9(24.2) | 1.2 | 31.4 |
| Lactose | 177 | 1518 (25) | 4.090 (0.333) | \_\_ | 275.2(24.6) | -169.7(50.3) | 0.9 | 51.5 |
| Milk component contents (g/kg) |
| Fat  | 279 | 36.69 (0.29) | -0.073 (0.0068) | \_\_ | \_\_ | \_\_ | 2.5 | 1.57 |
| Protein | 275 | 31.54 (0.14) | 0.0267 (0.0033) | \_\_ | 0.50 (0.23) | -2.01 (0.44) | 2.3 | 0.60 |
| Lactose | 173 | 47.78 (0.15) | 0.0091 (0.0021) | \_\_ | \_\_ | \_\_ | 2.2 | 0.43 |

Nexp = Number of experimental groups; Outlier = Observations with studentized residuals higher than 3 (or lower than -3); RMSE=Root mean square error after adjusting for the effect of experiment.

1Standard errors of the coefficient are reported in parentheses.

Interaction between NEL100 and MP67 was not significant for any of the variables studied.

Models were chosen based on AIC (see material and methods). All coefficients were significantly different from 0 at least at the level P<0.05.

**Supplementary Table S3. Effect of weighting the observations on model coefficients estimate: Milk yield response to change in NEL and MP contents as an example**

**Table S3** Model comparison for the milk yield response to changes in dietary NEL content (MJ/kg DM) and MP content (g/kg DM). The co-variables are mean centred on: NEL = 6.7 MJ/kg DM, MP = 100 g/kg DM.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Nexp | Intercept | Linear NEL | Quadratic NEL | Linear MP | Quadratic MP | NEL x MP | Outlier (%) | RMSE |
| Milk (kg/d) |
| Model 1 | 267 | 32.16 (0.09) | 0.81 (0.29) | -1.19 (0.33) | 0.098 (0.009) | -0.0031 (0.0004) | 0.061 (0.016) | 2.5 | 1.26 |
| Model 2 | 267 | 32.18 (0.10) | 0.87 (0.29) | -1.29 (0.34) | 0.102 (0.009) | -0.0031 (0.0004) | 0.059 (0.016) | 2.5 | 1.31 |
| Model 3 | 279 | 32.09 (0.10) | 0.99 (0.29) | -1.05 (0.34) | 0.104 (0.009) | -0.0028 (0.0003) | 0.050 (0.016) | 2.1 | 1.33 |
|  |  |  |  |  |  |  |  |  |  |

Nexp=Number of experimental groups; Outlier = Observations with studentized residuals higher than 3 (or lower than -3); RMSE=Root mean square error after adjusting for the effect of experiment.

1 Standard errors of the coefficient are reported in parentheses.

Model 1= Weighting of the observations with their reverse standard error of the mean (SEM) centred on the global average SEM, using only the dataset where SEM were reported (Nexp=272); Model 2= No weighting of the observations, using only the dataset where SEM were reported (Nexp=272); Model 3= No weighting of the observations, using the complete dataset (Nexp=282).

Standard errors of the coefficient are reported between brackets.

Models were chosen based on AIC (see material and methods). All predictions were significantly different from 0 at least at the level P<0.005.

**Supplementary Table S4. Main ingredient of the investigated treatments**

**Table S4** Main ingredients of the investigated treatments.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | Nt | Mean | SD | Minimum | Maximum |
| Ingredient, % of DM |
|  Maize silage  | 488 | 35.2 | 15.8 | 7.0 | 97.7 |
|  Alfalfa silage | 299 | 29.4 | 18.1 | 1.0 | 100.0 |
|  Grass silage | 205 | 53.8 | 19.6 | 7.0 | 100.0 |
|  Alfalfa hay | 193 | 23.1 | 15.5 | 3.5 | 81.2 |
|  Ground maize | 367 | 23.2 | 11.0 | 1.2 | 51.9 |
|  Maize gluten meal | 128 | 3.5 | 3.3 | 0.3 | 19.0 |
|  Maize gluten feed | 103 | 8.4 | 6.8 | 1.7 | 33.6 |
|  Dry distillers grains with solubles | 93 | 8.0 | 6.6 | 0.2 | 30.1 |
|  Wheat | 168 | 9.7 | 6.6 | 1.0 | 50.2 |
|  Barley  | 256 | 14.1 | 10.6 | 1.2 | 58.2 |
|  Beet pulp1 | 158 | 8.5 | 8.2 | 0.1 | 58.3 |
|  Molasses2 | 280 | 1.8 | 1.4 | 0.2 | 6.8 |
|  Fish meal | 136 | 2.0 | 1.7 | 0.2 | 12.9 |
|  Blood meal | 111 | 1.6 | 2.1 | 0.2 | 9.4 |
|  Soybean meal | 614 | 7.2 | 5.0 | 0.2 | 23.6 |
|  Soybean meal, rumen bypass | 162 | 6.5 | 4.4 | 0.3 | 20.1 |
|  Soybean hulls | 160 | 8.0 | 6.2 | 0.2 | 31.6 |
|  Rapeseed meal | 155 | 5.3 | 4.5 | 0.2 | 24.1 |
|  Fat supplements3 | 324 | 1.4 | 1.2 | 0.1 | 6.0 |
|  Urea | 187 | 0.56 | 0.45 | 0.03 | 3.70 |

Statistics on inclusion percentage of feed ingredients were performed only with the treatments for which the diet included the ingredient studied.

1Beet-pulp either molassed or unmolassed

2Molasses from either sugarcane or sugar beet.

3Fat supplements included oils from vegetables (maize, rapeseed, soybean and sunflower) or from fish or Ca-salt of fatty acids, tallow and yellow grease.