Net hepatic release of glucose from precursor supply in ruminants: a meta-analysis

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**SUPPLEMENTARY MATERIALS**

**Supplementary Material S1. Terminology on net nutrient fluxes**

Afferent flux: Flux of nutrient supplied to the liver via the portal vein and the hepatic artery

= (nutrient concentration in portal venous blood/plasma x portal venous blood/plasma flow) + (arterial nutrient concentration x hepatic arterial blood/plasma flow)

Efferent flux: Flux of nutrient released by the liver via the hepatic vein

= nutrient concentration in hepatic venous blood/plasma x hepatic venous blood/plasma flow

Net hepatic flux = efferent hepatic flux – afferent hepatic flux

The terminology ‘net hepatic uptake’ refers to a positive net hepatic flux, ie. afferent flux > efferent flux.

The terminology ‘net hepatic release’ refers to a negative net hepatic flux, ie. afferent flux < efferent flux.

**Supplementary Material S2. List of references used in the meta-analysis**

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**Supplementary Table S1.** *Calculation of endogenous metabolites mobilised when ruminants are in negative energy balance*

|  |
| --- |
| Calculation of energy balance§1 |
| ME requirements for maintenance (**MEm**, kJ/kg BW 0.75 per day)  = 481.5 for dairy cattle and beef cattle  = 439.6 for dry and gestating cattle and for sheep  When ME intake < MEm :  Energy Balance (**EB,** kJ /kg BW 0.75 per day) = (ME intake – MEm) × km  When ME intake > MEm :  Expected NE for production (**NEP**, kJ /kg BW 0.75 per day) = (ME intake - MEm) × k  Energy Balance (EB)  = expected NEP, for growing animals  = expected NEP – observed NEP, for lactating or gestating animals  Mobilized energy (kJ/kg BW 0.75 per day) = EB, when EB < 0 |
| Calculation of mobilized alanine§2 |
| Mobilized proteins (g/kg BW 0.75 per day) = (mobilized energy x 0.293) / 5.6  Mobilized alanine (moles of carbon/kg BW 0.75 per day) = (mobilized proteins / 89.1) x 4 |
| Calculation of mobilized glycerol§3 |
| Mobilized fat (g/kg BW 0.75 per day) = (mobilized energy x 3.89) / 9.2  Mobilized glycerol (moles of carbon/kg BW 0.75 per day) = [ (mobilized fat x 0.105) / 92] x 3 |

Table modified from Loncke C, Nozière P, Bahloul L, Vernet J, Lapierre H, Sauvant D and Ortigues-Marty I. 2015. Empirical prediction of net splanchnic release of ketogenic nutrients, acetate, butyrate and β-hydroxybutyrate in ruminants: A meta-analysis. Animal 9, 449-463. (reprinted with permission)

§1 ME= metabolisable energy; NE = net energy; GE = gross energy; km = 0.287 × ME/GE + 2.32 ; k = kl = 0.249 × ME/GE + 1.94 for lactation and moderate growth or k = kg = 0.13 for gestation ; observed NEP for lactation = 3.094 MJ/Lmilkper day, assuming a milk fat content at 4% (except in the 5 publications which reported milk fat content ranging from 3.6% to 4.5%), and NEP for gestation = 17.15 MJ/day for Holstein cows, 6.70 MJ/day for Dorset sheep and 10.7 MJ/day for Lacaune sheep. All values are based on INRA (2007)

§2 with 0.07 being the proportion of proteins in mobilized energy, 5.6 the energy density of proteins (MJ/g), 89.1 the molar mass of alanine (g/mol) and 4 the number of carbons in one mole of alanine (mole/mole)

§3 with 0.93 being the proportion of fat in mobilized energy, 9.2 the energy density of fat (MJ/g), 0.105 the proportion of glycerol in mobilized fat assuming that body fat is composed of 100% triglycerides and that one mole of triglyceride comprises 1 mole of glycerol and 3 moles of fatty acids (of 887 g/mole of average molar mass), 92 the molar mass of glycerol (g/mol), and 3 the number of carbons in one mole of glycerol (mole/mole)

**Supplementary Material S3.** **Influence of analytical methods on models**

Attention was paid to the methods used to determine net hepatic fluxesto ensure that all results could be combined in the meta-analysis. Methodological aspects considered were the selection of the matrix, blood or plasma, and of the analytical method for the analysis of metabolites, as well as the presence *vs*. absence of a deacetylation step in the analysis of para-aminohippuric acid used to determine blood or plasma flows.

*Measurement of metabolites on blood vs plasma.* In the eligible dataset, concentrations were mainly determined on blood (n = 49 and 4 for propionate, n = 76 and 25 for α-amino-N, n = 60 and 30 for L-lactate and n = 58 and 60 for glucose, in blood and plasma respectively). No results on total or individual amino acid fluxes were available. For propionate (data not shown) and α-amino-N (Martineau *et al*., 2009), no difference had been observed whether fluxes were measured in plasma or blood. For L-lactate, differences between blood and plasma fluxes depend on the nutritional status of the animals (Aufrère, 1979). For glucose, net portal appearance of glucose tended to be overestimated by plasma measurements when it was low, but underestimated up to 25 % with high intakes in ewes (Nozière *et al*., 1998). But no systematic correction factor exists for L-lactate and glucose. Hence all plasma and blood results were pooled. The lack of gross bias between plasma and blood results was only checked by graphical examination.

*Analytical methods to determine metabolite concentrations.* Analytical methods used to measure glucose (oxi-peroxydase), α-amino-N (ninhydrine) and L-lactate (L-lactate dehydrogenase) were similar among publications. Similarly, all but one (solvent extraction) selected publications indicated that propionate was analysed after an extraction by ion exchange resins. Consequently, no correction was applied to account for differences in analytical methods. It was checked that all blood or plasma concentrations of nutrients were superior to the analytical limits of quantification (Ortigues *et al*., 2003) determined in our laboratory (limit of quantification = 1.13 mM for glucose, 0.19 mM for propionate and 0.32 mM for α-amino-N, not defined for L-lactate). All data met this analytical reliability criterion.

*Impact of para-aminohippuric acid determination method*. Hepatic blood flows were measured using the para-aminohippuric acid (**pAH**) down-stream dilution method. 86 % of all publications did not correct for the incomplete recovery of pAH across the liver, demonstrated in sheep by Katz and Bergman (1969) and in cows by Kristensen *et al*. (2009) and Rodriguez-Lopez *et al.* (2014). To evaluate the potential impact of this methodological error, it was tested whether correction of net hepatic fluxes for incomplete marker recovery modified the prediction equations. Net fluxes were recalculated assuming that the hepatic arterial blood/plasma flow represented 10% of hepatic venous blood/plasma flow as measured when pAH is deacetylated before analysis (Rodriguez-Lopez *et al.*, 2014). This correction could only be applied when publications reported blood flow and nutrient arterial concentration data in addition to net fluxes. It was the case in 77, 68, 67 and 88 % of the publications used in the models of glucose, propionate, lactate and α-amino-N, respectively.

Correcting net flux values for incomplete recovery of pAH could only be done for a limited number of publications. This limited dataset did not span over the whole meta-design and reduced the proportion of data on lactation and gestation, explaining why some uncorrected response equations established on this partial dataset (Supplementary Table S2) were significantly different from those reported in Tables 4 and 5. Correcting net flux values for pAH acetylation did not modify net hepatic uptake of propionate, increased net uptakes of L-lactate and reduced that of α-amino-N and glucose. Values averaged 0.679±0.053 *vs.* 0.680±0.053, 0.276±0.031 *vs.* 0.294±0.030, 0.393±0.033 *vs.* 0.353±0.025, and 0.712±0.037 *vs.* 0.695±0.038 mmol/ kg BW per hour, for uncorrected and corrected fluxes, in the same respective order of nutrient. The marginal rates of nutrient uptake by the liver were significantly reduced for L-lactate and α-amino-N only, as well as the marginal rate of glucose release from available precursors. Intercept values remained not significantly different from zero, changes in their numerical values reflected changes in net fluxes reported above.

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**Supplementary Table S2** *Response models of net hepatic fluxes (NHF, mmol/kg BW per hour) of propionate (C3), L-lactate, α-amino-N (*αN*) and glucose (mmol C/kg BW per hour) after correction of hepatic blood flows for incomplete para-aminohippuric (pAH) recovery in ruminants. Since corrections could not be applied to the full dataset, uncorrected and corrected values are shown for the sub-datasets used*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | pAH acid uncorrected values | | | pAH corrected values | | |
| Models | nexp | Equations | RMSE | *R2adj* |  | RMSE | *R2adj* |
| NHF-C3 | 21 | 0.0646NSa±0.0345 - 0.9896\*\*b±0.0443 x NPA-C3 | 0.0275 | 0.99 | 0.0541NSc±0.0291 - 0.9812\*\*\*c±0.0374 x NPA-C3 | 0.0232 | 0.99 |
| NHF-L-lactate | 12 | -0.2235NSa±0.1067 + 0.2322\*\*a±0.1313 x NPA-C3  - 1.0677\*\*\*a±0.1244 x NPA-L-lactate | 0.0613 | 0.91 | -0.1873NSc±0.0817 + 0.1379\*\*d±0.1006 x NPA-C3  - 0.9904\*\*\*d±0.0954 x NPA-L-lactate | 0.0469 | 0.94 |
| NHF-αN | 23 | -0.0431NSb±0.0482 - 0.5819\*\*b±0.0879 x NPA-αN | 0.0805 | 0.90 | -0.0891NSd±0.0483 - 0.4615\*d±0.088 x NPA- αN | 0.0808 | 0.83 |
| NHF-glucose | 30 | -0.7407NSa±0.8820 + 0.7971\*\*a±0.1233 x NPA-prec | 0.4352 | 0.90 | 0.495NSc±1.496 + 0.6824\*\*c±0.2092 x NPA-prec | 0.738 | 0.81 |

nexp = number of experimental groups in the model; RMSE = Residual means square error; *R2adj*= adjusted *R2*; NS: non significantly different from zero; \* *P*<0.05; \*\* *P*<0.01; \*\*\* *P*<0.001; NPA = net portal appearance; prec = glucose precursors

a No significant difference with the same parameter calculated from the whole dataset

b Significant difference with the same parameter calculated from the whole dataset

c No significant difference with the same parameter calculated from the pAH-uncorrected sub-dataset (*P*>0.05)

d Significant difference with the same parameter calculated from the pAH-uncorrected sub-dataset (*P*<0.05)

**Supplementary Material S4.** **Influence of animal profile**

*Influence of physiological status*

Non-productive adults (56% of the data) were characterised by DM intake ranging from fasting to 41 g/kg BW. Growing animals (21% of the data) and lactating cows were fed diets rich in concentrate (47-48%, P<0.03 compared to other physiological statuses). All lactating animals (15% of the data) were dairy cows between 11 and 240 days in milk (13 treatments for the first 80 days in milk, and 10 for 120 to 240 days in milk). They were fed the highest levels of DM intake (*P*=0.049) compared to animals in other physiological status. Data on gestating animals (8% of the data; all in late gestation, ≤ 2 months before calving) were limited, but sufficient to be included in some analyses.

*Influence of animal species*

For each nutrient, the distribution of treatments was similar between cattle and sheep and between females and males (data not shown). Among available data, cattle and sheep had similar average DM intake per kg BW (*P*=0.47) but diets fed to sheep had different composition with lower proportions of concentrate (*P*<0.001) and lower digestibility (*P*<0.001) compared with cattle. Consequently, dietary intakes differed between cattle and sheep for all constituents except for dietary rumen fermentable organic matter intake (*P*=0.60) (data not shown).

**Supplementary Table S3.a** *Description of diets used for the meta-analyses1 of net hepatic fluxes of propionate, L-lactate, α-amino-nitrogen and glucose according to species (cattle and sheep)*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cattle | | | | |  | Sheep | | | | | |  | Species effect |
|  | nt | mean | SD | Min | Max |  | nt | mean | SD | Min | | Max |  | P value |
| Dietary composition2 (g/kg DM) | | | | | | | | | | | | | | |
| Crude fiber | 62 | 171 | 71.7 | 74.6 | 371 |  | 95 | 273 | 83.7 | 39.5 | 404 | |  | < 0.001 |
| NDF | 62 | 352 | 122 | 170 | 710 |  | 95 | 522 | 137 | 133 | 712 | |  | < 0.001 |
| ADF | 62 | 188 | 75.9 | 87.7 | 403 |  | 95 | 296 | 86.7 | 43.8 | 393 | |  | < 0.001 |
| Starch | 62 | 52.6 | 36.3 | 0.0 | 110 |  | 95 | 21.3 | 30.0 | 0.0 | 106 | |  | < 0.001 |
| CP | 62 | 152 | 29.9 | 85.0 | 247 |  | 95 | 122 | 33.2 | 44.7 | 181 | |  | < 0.001 |
| Digestible OM | 62 | 710 | 65.7 | 478 | 793 |  | 95 | 604 | 85.3 | 458 | 852 | |  | < 0.001 |
| Digestible NDF | 62 | 216 | 83.2 | 97.3 | 403 |  | 95 | 297 | 92.1 | 94.9 | 474 | |  | < 0.001 |
| Digestible CP | 62 | 102 | 27.0 | 39.0 | 166 |  | 95 | 72.9 | 30.8 | 4.90 | 135 | |  | < 0.001 |
| Rumen fermentable OM | 62 | 508 | 41.4 | 436 | 631 |  | 95 | 500 | 43.7 | 413 | 586 | |  | 0.238 |
| Rumen digestible NDF | 62 | 195 | 74.8 | 87.7 | 363 |  | 95 | 268 | 82.9 | 85.4 | 427 | |  | < 0.001 |
| Rumen fermentable CP | 62 | 92.1 | 26.8 | 45.9 | 167 |  | 95 | 72.5 | 25.1 | 27.6 | 128 | |  | < 0.001 |
| PDI | 62 | 95.6 | 14.9 | 54.3 | 126 |  | 95 | 77.5 | 19.8 | 26.9 | 111.5 | |  | < 0.001 |
| ME (MJ/kg DM) | 62 | 11.1 | 1.15 | 7.03 | 12.5 |  | 95 | 9.20 | 1.42 | 6.75 | 13.1 | |  | < 0.001 |
| Proportion of concentrate  (g/100 g DM) | 62 | 54.1 | 30.4 | 0.0 | 100 |  | 95 | 25.0 | 33.6 | 0.0 | 100 | |  | < 0.001 |
|  |  |  |  |  |  |  |  |  |  |  |  | |  |  |
| Intake (g/kg BW per day) |  |  |  |  |  |  |  |  |  |  |  | |  |  |
| DM | 62 | 21.0 | 5.90 | 0.0 | 36.9 |  | 95 | 22.1 | 8.92 | 0.0 | 47.5 | |  | 0.378 |
| Digestible OM | 62 | 14.9 | 4.51 | 0.0 | 28.6 |  | 95 | 13.4 | 5.47 | 0.0 | 30.0 | |  | < 0.001 |
| Rumen fermentable OM | 62 | 10.7 | 3.26 | 0.0 | 17.4 |  | 95 | 11.1 | 4.61 | 0.0 | 25.4 | |  | 0.540 |
| Rumen digestible NDF | 62 | 4.07 | 1.74 | 0.0 | 7.53 |  | 95 | 6.03 | 3.23 | 0.0 | 14.3 | |  | < 0.001 |
| Starch | 62 | 5.60 | 3.68 | 0.0 | 16.1 |  | 95 | 2.70 | 3.90 | 0.0 | 15.2 | |  | < 0.001 |
|  |  |  |  |  |  |  |  |  |  |  |  | |  |  |
| Energy balance  (kJ/kg BW per day) | 62 | 29.9 | 43.0 | -70.9 | 88.9 |  | 95 | 11.2 | 61.0 | -173 | 109.4 | |  | < 0.001 |

Min = minimum value; Max = maximum value; nt = number of treatments

1 see Supplementary Material S1

2 Dietary composition and intake calculated by additivity according to INRA Feed Tables (INRA, 2007); DM = dry matter; OM = organic matter; ME = metabolizable energy; PDI = protein digestible in the intestine; BW = body weight.

**Supplementary Table S3.b** *Description of arterial concentrations, net portal appearance, net hepatic fluxesa and estimated potential contribution to neoglucognenesis of propionate, L-lactate, α-amino-N (α-N) and glucose used for the meta analysisb of net hepatic fluxes of propionate, L-lactate, α-amino-N and glucose according to species (cattle and sheep)*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cattle | | | | |  | Sheep | | | | |  | Species Effect |
|  | nt | mean | SD | Min | Max |  | nt | mean | SD | Min | Max |  | *P-*value |
| Arterial concentration (mM) | | | | | | | | | | | | | |
| Propionate | 26 | 0.0608 | 0.0186 | 0.031 | 0.096 |  | 24 | 0.0334 | 0.0348 | 0.012 | 0.160 |  | 0.001 |
| α-amino-N | 24 | 3.29 | 1.07 | 2.27 | 7.19 |  | 51 | 4.017 | 1.017 | 0.0 | 6.10 |  | 0.006 |
| L-lactate | 40 | 0.493 | 0.145 | 0.200 | 0.756 |  | 29 | 0.842 | 0.344 | 0.340 | 1.42 |  | < 0.001 |
| Glucose | 43 | 4.035 | 0.645 | 2.73 | 5.49 |  | 49 | 3.29 | 0.546 | 1.89 | 4.56 |  | < 0.001 |
| Insulin (μUI/L) | 12 | 21.5 | 13.7 | 6.31 | 51.47 |  | 7 | 38.9 | 23.0 | 18.4 | 71.9 |  | 0.520 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Net portal appearance (mmol/kg BW per hour) | | | | | | | | | | | | | |
| Propionate | 40 | 0.904 | 0.285 | 0.447 | 1.78 |  | 35 | 0.589 | 0.40 | 0.085 | 2.11 |  | < 0.001 |
| α-amino-N | 28 | 0.358 | 0.174 | 0.091 | 0.711 |  | 67 | 0.577 | 0.405 | 0.0 | 2.71 |  | 0.007 |
| L-lactate | 44 | 0.250 | 0.091 | 0.074 | 0.403 |  | 42 | 0.250 | 0.167 | 0.084 | 0.767 |  | 0.41 |
| Glucose | 52 | -0.022 | 0.118 | -0.234 | 0.352 |  | 62 | -0.084 | 0.107 | -0.373 | 0.168 |  | 0.004 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Net hepatic flux (mmol/kg BW per hour) | | | | | | | | | | | | | |
| Propionate | 34 | -0.827 | 0.269 | -1.69 | -0.409 |  | 33 | -0.494 | 0.256 | -1.10 | -0.085 |  | < 0.001 |
| α-amino-N | 22 | -0.215 | 0.105 | -0.513 | -0.033 |  | 69 | -0.461 | 0.263 | -1.30 | 0.436 |  | < 0.001 |
| L-lactate | 46 | -0.236 | 0.292 | -1.94 | -0.107 |  | 44 | -0.314 | 0.237 | -0.870 | 0.029 |  | 0.168 |
| Glucose | 52 | 0.763 | 0.327 | 0.317 | 1.40 |  | 72 | 0.639 | 0.312 | 0.0 | 1.74 |  | 0.034 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Estimated potential contribution to neoglucogenesis (%) | | | | | | | | | | | | | |
| Propionate | 34 | 61.5 | 11.8 | 44.5 | 94.7 |  | 35 | 44.8 | 22.4 | 12.3 | 99.7 |  | < 0.001 |
| α-amino-N | 28 | 27.4 | 9.55 | 14.2 | 43.6 |  | 39 | 48.1 | 26.7 | 0.0 | 179 |  | 0.004 |
| L-lactate | 16 | 18.2 | 13.3 | 1.23 | 85.4 |  | 49 | 34.8 | 29.5 | 0.10 | 149 |  | 0.001 |

Min = minimum value; Max = maximum value; nt = number of treatments

a a positive value indicates a net release; a negative value indicates a net uptake

b See Supplementary Material S1.

**Supplementary Material S5.** **Comparison of net hepatic uptake of nutrients predicted using net portal appearance of total afferent fluxes as the predictor**

**Supplementary Table S4.** *Response equations of the net hepatic fluxes (mmol/kgBW per hour) of propionate, α-amino-nitrogen, and L-lactate to variations in their net portal appearance (NPA, mmol/kgBW per hour) or total hepatic afferent flux (THAF, mmol/kgBW per hour) in ruminants*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Y | Number | |  | Equation |  | Adjustment | |
|  | nexp | nt |  |  |  | RMSE | Adjusted R² |
| Propionate | 27 | 69 |  | 0.0024 ± 0.021NS - 0.913 ± 0.027\*\*\*× NPA-propionate |  | 0.027 | 0.992 |
|  | 19 | 48 |  | 0.091 ± 0.042\* - 0.887 ± 0.049\*\*\*× THAF-propionate |  | 0.003 | 0.990 |
| α-N | 30 | 85 |  | 0.0055 ± 0.033NS - 0.749 ± 0.067 \*\*\* × NPA-α-amino-nitrogen |  | 0.062 | 0.898 |
|  | 20 | 50 |  | -0.025 ± 0.175NS - 0.029 ± 0.157† × THAF-α-amino-nitrogen |  | 0.127 | 0.609 |
| L-lactate | 25 | 66 |  | -0.066 ± 0.026\* - 0.887 ± 0.105\*\*\* × NPA-L-lactate |  | 0.071 | 0.880 |
|  | 20 | 52 |  | -0.169 ± 0.097† - 0.067 ± 0.051NS × THAF-L-lactate |  | 0.126 | 0.677 |

NS: not significant (*P*>0.10); † *P*<0.10; \* *P*<0.05; \*\*\* *P*<0.001; nexp: number of experimental groups in the model; nt: number of treatments in the model; RMSE = residual means square error

**Supplementary Material S6. LSMeans study**

**Supplementary Table S5.** Linear relationships between least square means (LSMeans, Y variable, mmol/h per kg BW) and interfering factors (X variable) detected in the models listed in Tables 3 and 4, in ruminants

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Model number** | **Y variable, LSMeans of** | **X Variable** | **Intercept** | | **Linear term** | | **RMSE** | Adjusted **R²** |
|  |  |  | **α** | **SD** | **β** | **SD** |  |  |
| 1 | NHF-C3 | EB  (kJ/d/kg BW) | -2,824\*\*\* | 0.251 | 0.0015\*\* | 0.0005 | 0.113 | 0.245 |
| 1 | NHF-C3 | Starch intake (g/j/kg BW) | -0.684\*\*\* | 0.008 | 0.0004\* | 0.0002 | 0.029 | 0.015 |
| 2 | NHF-aN | CP (g/kg DM) | -0.621\*\*\* | 0.119 | 0.0020\* | 0.0009 | 0.114 | 0.132 |
| 2 | NHF-aN | PDIE (g/kg DM) | -0.725\*\*\* | 0.148 | 0.0039\* | 0.0016 | 0.112 | 0.169 |
| 2 | NHF-aN | PDIN (g/kg DM) | -0.583\*\*\* | 0.001 | 0.0024\* | 0.0011 | 0.115 | 0.122 |
| 2 | NHF-aN | NHF-glucose (mmol/h/kg BW) | -0.475\*\*\* | 0.061 | 0.2027\* | 0.0918 | 0.109 | 0.156 |
| 3 | NHF-L-lactate | NHF-C3 (mmol/h/kg BW) | -0.470\*\*\* | 0.058 | -0.2349\*\* | 0.0773 | 0.087 | 0.326 |
| 3 | NHF-L-lactate | NPA-glucose (mmol/h/kg BW) | -0.229\*\*\* | 0.030 | 0.6704\* | 0.2646 | 0.112 | 0.184 |
| 3 | NHF-L-lactate | Starch intake (g/d/kg BW) | -0.353\*\*\* | 0.035 | 0.0168\* | 0.0068 | 0.111 | 0.171 |
| 3 | NHF-L-lactate | ME (MJ /kg DM) | -3.230\*\*\* | 0.699 | 0.0002\*\* | 0.0001 | 0.448 | 0.234 |
| 3 | NHF-L-lactate | DOM (g/kg DM) | -0.899\*\*\* | 0.181 | 0.0009\*\* | 0.0003 | 0.102 | 0.299 |
| 5 | NHF-L-lactate | NPA-glucose (mmol/h/kg BW) | -0.191\*\*\* | 0.048 | 1.3195\*\* | 0.4137 | 0.167 | 0.285 |
| 5 | NHF-L-lactate | NPA-N (mmol/h/kg BW) | -0.044 NS | 0.094\*\* | -0.6733 | 0.2110 | 0.170 | 0.338 |
| 7 | NHF-glucose | NPA\_N (mmol/h/kg BW) | 2.378\*\*\* | 0.585 | 3.6750\*\* | 1.1770 | 0.866 | 0.368 |
| 7 | NHF-glucose | NHF-BHB (mmol/h/kg BW) | 2.727\*\*\* | 0.263 | 4.4347\*\*\* | 0.8642 | 0.571 | 0.613 |
| 11 | NHF-glucose | ME (MJ/kg DM) | -2.014NS | 1.398 | 0.5619\*\*\* | 0.1377 | 1.046 | 0.335 |
| 13 | NHF-glucose | NHF-BHB | 3.269\*\*\* | 0.249 | 0.378\* | 0.185 | 0.597 | 0.131 |

NS = not significant; \* P<0.05; \*\*P<0.01; \*\*\*P<0.001

aN α-amino-N; BW body weight; BHB β-hydroxybutyrate; C3 propionate; DM dry matter; DOM digestible organic matter; EB energy balance; ME metabolisable energy; NHF net hepatic flux; NPA net portal appearance; PDIE(N) protein digestible in the intestine as limited by the energy (nitrogen) supply; RMSE residual mean square error