

## Supplementary data

**Table S1:** Average concentrations of total metabolites ( $\mu\text{M}$ ) for skim milk powder derived from perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR) feeding systems, determined by LC-MS/MS.

<sup>a,b</sup> indicates values within a row not sharing a common superscript letter differed significantly ( $p < 0.05$ ).

\* denotes where a replicate was below the limit of detection or limit of quantification.

Metabolite ( $\mu\text{M}$ )	GRS	CLV	TMR
Acetylmethionine	0.97 ( $\pm$ 0.07)	0.60 ( $\pm$ 0.08)	0.88 ( $\pm$ 0.54)
Alanine	34.1 ( $\pm$ 2.90)	30.2 ( $\pm$ 3.32)	41.9 ( $\pm$ 1.20)
alpha-Aminoadipic acid	6.86 ( $\pm$ 0.47)	5.45 ( $\pm$ 2.26)	7.42 ( $\pm$ 1.83)
Arginine	14.2 ( $\pm$ 0.42)	14.6 ( $\pm$ 1.70)	16.6 ( $\pm$ 0.14)
Asparagine	1.41 ( $\pm$ 0.84)	2.11*	1.29 ( $\pm$ 0.16)
Aspartic acid	10.2 ( $\pm$ 3.34)	17.3 ( $\pm$ 4.38)	11.5 ( $\pm$ 2.38)
Asymmetric dimethylarginine	0.09 ( $\pm$ 0.09)	0.05 ( $\pm$ 0.03)	0.09 ( $\pm$ 0.01)
Betaine	74.2 ( $\pm$ 20.01)	61.0 ( $\pm$ 19.30)	94.2 ( $\pm$ 12.52)
Carnosine	0.35 ( $\pm$ 0.02)	0.40 ( $\pm$ 0.04)	0.47 ( $\pm$ 0.08)
Choline	1245 ( $\pm$ 190.92)	1024 ( $\pm$ 319.61)	1035 ( $\pm$ 35.36)
cis-4-Hydroxyproline	0.31 ( $\pm$ 0.00)	0.34 ( $\pm$ 0.01)	0.32 ( $\pm$ 0.01)
Citrulline	1.89 ( $\pm$ 0.16)	1.71 ( $\pm$ 0.94)	2.29 ( $\pm$ 0.47)
Creatine	512 ( $\pm$ 7.07)	507 ( $\pm$ 4.24)	567 ( $\pm$ 65.76)
Creatinine	111 ( $\pm$ 2.83)	93.1 ( $\pm$ 14.00)	92.6 ( $\pm$ 3.82)
Diacetylspermine	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)
Dihydroxyphenylalanine	0.18 ( $\pm$ 0.02)	0.16 ( $\pm$ 0.00)	0.15 ( $\pm$ 0.02)
Dopamine	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)
Glutamic acid	256 ( $\pm$ 7.07)	284 ( $\pm$ 12.73)	241 ( $\pm$ 7.07)
Glutamine	4.38 ( $\pm$ 0.54) <sup>a</sup>	4.95 ( $\pm$ 1.24) <sup>a</sup>	12.4 ( $\pm$ 0.28) <sup>b</sup>
Glycine	65.0 ( $\pm$ 28.35)	54.3 ( $\pm$ 13.86)	74.1 ( $\pm$ 16.55)
Histidine	2.35 ( $\pm$ 0.57)	2.37 ( $\pm$ 0.42)	3.41 ( $\pm$ 0.43)
Isoleucine	2.49 ( $\pm$ 0.62)	2.40 ( $\pm$ 0.03)	4.32 ( $\pm$ 0.23)
Leucine	2.67 ( $\pm$ 1.14)	3.10 ( $\pm$ 0.95)	5.59 ( $\pm$ 1.20)
Lysine	15.8 ( $\pm$ 2.05)	13.8 ( $\pm$ 1.48)	12.9 ( $\pm$ 0.28)
Methionine	0.66 ( $\pm$ 0.03)	0.71 ( $\pm$ 0.18)	0.42 ( $\pm$ 0.08)
Methionine sulfoxide	0.07*	0.00*	0.22 ( $\pm$ 0.13)
Methylhistidine	1.20 ( $\pm$ 0.15)	0.99 ( $\pm$ 0.07)	1.00 ( $\pm$ 0.07)
Ornithine	2.24 ( $\pm$ 0.68)	2.11 ( $\pm$ 0.87)	4.14 ( $\pm$ 0.57)
Phenylalanine	1.44 ( $\pm$ 0.01)	1.19 ( $\pm$ 0.06)	1.42 ( $\pm$ 0.00)
Phosphocreatine	8.05 ( $\pm$ 1.70) <sup>a,b</sup>	6.32 ( $\pm$ 0.56) <sup>a</sup>	16.3 ( $\pm$ 4.45) <sup>b</sup>
Proline	15.3 ( $\pm$ 0.71)	14.0 ( $\pm$ 0.64)	19.1 ( $\pm$ 1.41)
Putrescine	0.07 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.01)	0.04 ( $\pm$ 0.00)
Sarcosine	0.48 ( $\pm$ 0.05)	0.56 ( $\pm$ 0.03)	0.42 ( $\pm$ 0.09)
Serine	22.1 ( $\pm$ 0.78) <sup>b</sup>	18.3 ( $\pm$ 1.20) <sup>a,b</sup>	10.0 ( $\pm$ 0.55) <sup>a</sup>
Serotonin	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)
Spermidine	0.83 ( $\pm$ 0.13)	0.77 ( $\pm$ 0.06)	0.82 ( $\pm$ 0.03)

Spermine	0.48 (± 0.05)	0.50 (± 0.13)	0.59 (± 0.02)
Symmetric dimethylarginine	4.36 (± 0.28)	3.26 (± 0.24)	3.38 (± 0.87)
Taurine	26.5 (± 2.69)	25.0 (± 1.84)	30.3 (± 1.84)
Threonine	4.49 (± 0.61)	3.82 (± 0.35)	4.99 (± 0.67)
Total dimethylarginine	4.45 (± 0.19)	3.31 (± 0.21)	3.47 (± 0.86)
trans-4-Hydroxyproline	4.39 (± 0.04)	4.25 (± 0.15)	4.53 (± 0.22)
Trimethylamine N-oxide	3.35 (± 0.69)	2.83 (± 0.08)	3.22 (± 0.02)
Tryptophan	0.83 (± 0.02)	0.74 (± 0.06)	0.83 (± 0.11)
Tyramine	0.01*	0.01 (± 0.01)	0.00 (± 0.00)
Tyrosine	0.31 (± 0.01)	0.28 (± 0.08)	0.64 (± 0.02)
Valine	7.49 (± 0.12) <sup>a</sup>	7.27 (± 1.09) <sup>a</sup>	11.3 (± 1.34) <sup>b</sup>

**Table S2:** Average concentrations of total metabolites ( $\mu\text{M}$ ) for sweet whey powder derived from perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR) feeding systems, determined by LC-MS/MS.

<sup>a,b</sup> indicates values within a row not sharing a common superscript letter differed significantly ( $p < 0.05$ ).

\* denotes where a replicate was below the limit of detection or limit of quantification.

Metabolite ( $\mu\text{M}$ )	GRS	CLV	TMR
Acetylorntine	1.26 ( $\pm$ 0.25)	0.87 ( $\pm$ 0.15)	0.80 ( $\pm$ 0.04)
Alanine	44.6 ( $\pm$ 7.64)	35.2 ( $\pm$ 2.90)	39.2 ( $\pm$ 5.30)
alpha-Aminoadipic acid	10.6 ( $\pm$ 3.15)	9.32 ( $\pm$ 2.67)	7.69 ( $\pm$ 1.15)
Arginine	16.0 ( $\pm$ 2.90)	14.7 ( $\pm$ 0.00)	14.9 ( $\pm$ 0.64)
Asparagine	1.25*	1.70*	3.24 ( $\pm$ 1.13)
Aspartic acid	10.5 ( $\pm$ 0.28)	18.7 ( $\pm$ 11.31)	10.2 ( $\pm$ 0.66)
Asymmetric dimethylarginine	0.18 ( $\pm$ 0.06)	0.04 ( $\pm$ 0.02)	0.06 ( $\pm$ 0.04)
Betaine	80.7 ( $\pm$ 16.48)	63.9 ( $\pm$ 23.05)	73.0 ( $\pm$ 19.09)
Carnosine	0.40 ( $\pm$ 0.06)	0.39 ( $\pm$ 0.04)	0.48 ( $\pm$ 0.08)
Choline	1415 ( $\pm$ 219.20)	1224 ( $\pm$ 574.17)	892 ( $\pm$ 1.41)
cis-4-Hydroxyproline	0.34 ( $\pm$ 0.00)	0.33 ( $\pm$ 0.01)	0.32 ( $\pm$ 0.01)
Citrulline	1.50 ( $\pm$ 0.28)	1.38 ( $\pm$ 1.20)	2.46 ( $\pm$ 0.69)
Creatine	761 ( $\pm$ 108.19)	543 ( $\pm$ 26.16)	561 ( $\pm$ 101.82)
Creatinine	115 ( $\pm$ 14.85)	93.6 ( $\pm$ 6.93)	72.9 ( $\pm$ 5.59)
Diacetylspermine	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)
Dihydroxyphenylalanine	0.21 ( $\pm$ 0.02)	0.17 ( $\pm$ 0.02)	0.17 ( $\pm$ 0.00)
Dopamine	0.08 ( $\pm$ 0.00)	0.08*	0.08 ( $\pm$ 0.00)
Glutamic acid	318 ( $\pm$ 46.67)	297 ( $\pm$ 33.23)	238 ( $\pm$ 9.19)
Glutamine	1.36 <sup>*a</sup>	0.187 ( $\pm$ 0.02) <sup>a</sup>	7.02 ( $\pm$ 3.20) <sup>b</sup>
Glycine	93.9 ( $\pm$ 55.37)	61.7 ( $\pm$ 1.13)	47.8 ( $\pm$ 11.17)
Histidine	2.61 ( $\pm$ 0.74)	2.50 ( $\pm$ 0.16)	3.11 ( $\pm$ 0.53)
Isoleucine	3.10 ( $\pm$ 0.71)	2.49 ( $\pm$ 0.39)	3.81 ( $\pm$ 0.28)
Leucine	3.20 ( $\pm$ 0.48)	3.24 ( $\pm$ 0.24)	4.16 ( $\pm$ 2.14)
Lysine	18.6 ( $\pm$ 2.90)	14.0 ( $\pm$ 2.12)	12.5 ( $\pm$ 1.13)
Methionine	1.34 ( $\pm$ 0.08)	1.23 ( $\pm$ 0.04)	0.95 ( $\pm$ 0.14)
Methionine sulfoxide	0.42 ( $\pm$ 0.14)	0.47 ( $\pm$ 0.06)	0.25 ( $\pm$ 0.31)
Methylhistidine	1.26 ( $\pm$ 0.25)	1.13 ( $\pm$ 0.28)	0.92 ( $\pm$ 0.04)
Ornithine	2.89 ( $\pm$ 1.20)	1.92 ( $\pm$ 0.03)	2.76 ( $\pm$ 0.28)
Phenylalanine	1.72 ( $\pm$ 0.08)	1.51 ( $\pm$ 0.06)	1.46 ( $\pm$ 0.02)
Phosphocreatine	9.65 ( $\pm$ 0.93) <sup>a</sup>	6.49 ( $\pm$ 0.70) <sup>a</sup>	22.6 ( $\pm$ 6.01) <sup>b</sup>
Proline	16.8 ( $\pm$ 2.33)	15.5 ( $\pm$ 0.07)	18.3 ( $\pm$ 0.49)
Putrescine	0.06 ( $\pm$ 0.01)	0.06 ( $\pm$ 0.02)	0.04 ( $\pm$ 0.04)
Sarcosine	0.54 ( $\pm$ 0.21)	0.47 ( $\pm$ 0.17)	0.33 ( $\pm$ 0.03)
Serine	25.3 ( $\pm$ 3.11) <sup>b</sup>	18.7 ( $\pm$ 2.33) <sup>a,b</sup>	9.31 ( $\pm$ 0.64) <sup>a</sup>
Serotonin	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)
Spermidine	0.34 ( $\pm$ 0.04)	0.35 ( $\pm$ 0.04)	0.30 ( $\pm$ 0.00)
Spermine	0.05 ( $\pm$ 0.00)	0.06 ( $\pm$ 0.03)	0.08 ( $\pm$ 0.01)
Symmetric	4.60 ( $\pm$ 0.95)	3.30 ( $\pm$ 0.63)	3.06 ( $\pm$ 0.39)

dimethylarginine			
Taurine	29.8 (± 6.36)	28.4 (± 0.49)	30.5 (± 1.06)
Threonine	5.19 (± 1.20)	3.41 (± 0.50)	4.41 (± 0.18)
Total dimethylarginine	4.79 (± 1.01)	3.35 (± 0.64)	3.12 (± 0.43)
trans-4-Hydroxyproline	4.84 (± 0.30)	4.45 (± 0.17)	4.41 (± 0.37)
Trimethylamine N-oxide	3.33 (± 0.41)	3.19 (± 0.01)	3.32 (± 0.35)
Tryptophan	0.89 (± 0.35)	0.79 (± 0.01)	0.70 (± 0.10)
Tyramine	0.01 (± 0.00)	0.01 (± 0.00)	0.01 (± 0.00)
Tyrosine	0.42 (± 0.26)	0.28 (± 0.02)	0.51 (± 0.22)
Valine	8.50 (± 2.83)	7.78 (± 0.72)	10.6 (± 0.00)

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**Table S3:** Average concentrations of total metabolites ( $\mu\text{M}$ ) for ideal whey powder derived from perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR) feeding systems, determined by LC-MS/MS.

<sup>a,b</sup> indicates values within a row not sharing a common superscript letter differed significantly ( $p < 0.05$ ).

\* denotes where a replicate was below the limit of detection or limit of quantification.

Metabolite ( $\mu\text{M}$ )	GRS	CLV	TMR
Acetylorntine	0.96 ( $\pm$ 0.30)	0.98 ( $\pm$ 0.22)	0.78 ( $\pm$ 0.07)
Alanine	36.9 ( $\pm$ 4.03)	33.0 ( $\pm$ 1.84)	42.3 ( $\pm$ 4.03)
alpha-Aminoadipic acid	7.56 ( $\pm$ 1.16)	6.01 ( $\pm$ 2.51)	6.00 ( $\pm$ 0.57)
Arginine	13.4 ( $\pm$ 1.20)	13.8 ( $\pm$ 0.21)	15.6 ( $\pm$ 0.21)
Asparagine	1.45 ( $\pm$ 0.37)	0.34*	3.18 ( $\pm$ 0.95)
Aspartic acid	11.6 ( $\pm$ 0.99)	10.8 ( $\pm$ 2.93)	15.2 ( $\pm$ 2.19)
Asymmetric dimethylarginine	0.07 ( $\pm$ 0.03)	0.06 ( $\pm$ 0.02)	0.15 ( $\pm$ 0.01)
Betaine	85.5 ( $\pm$ 17.96)	66.8 ( $\pm$ 9.90)	83.8 ( $\pm$ 25.81)
Carnosine	0.38 ( $\pm$ 0.04)	0.37 ( $\pm$ 0.00)	0.43 ( $\pm$ 0.01)
Choline	1225 ( $\pm$ 120.21)	1020 ( $\pm$ 282.84)	906 ( $\pm$ 19.80)
cis-4-Hydroxyproline	0.33 ( $\pm$ 0.01)	0.31 ( $\pm$ 0.01)	0.32 ( $\pm$ 0.01)
Citrulline	1.40 ( $\pm$ 0.25)	1.67 ( $\pm$ 0.13)	2.66 ( $\pm$ 0.16)
Creatine	513 ( $\pm$ 29.70)	519 ( $\pm$ 12.02)	500 ( $\pm$ 55.86)
Creatinine	125 ( $\pm$ 12.02)	107 ( $\pm$ 17.47)	101 ( $\pm$ 8.84)
Diacetylspermine	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)
Dihydroxyphenylalanine	0.18 ( $\pm$ 0.01)	0.18 ( $\pm$ 0.01)	0.15 ( $\pm$ 0.00)
Dopamine	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)
Glutamic acid	266 ( $\pm$ 27.58)	269 ( $\pm$ 70.00)	282 ( $\pm$ 26.87)
Glutamine	4.04 ( $\pm$ 0.23) <sup>a</sup>	3.19 ( $\pm$ 1.77) <sup>a</sup>	11.5 ( $\pm$ 2.64) <sup>b</sup>
Glycine	66.8 ( $\pm$ 15.49)	61.2 ( $\pm$ 5.37)	56.4 ( $\pm$ 7.85)
Histidine	2.35 ( $\pm$ 0.50)	2.39 ( $\pm$ 0.45)	3.02 ( $\pm$ 0.17)
Isoleucine	2.76 ( $\pm$ 0.08)	2.78 ( $\pm$ 0.44)	3.86 ( $\pm$ 0.38)
Leucine	2.56 ( $\pm$ 1.63)	2.68 ( $\pm$ 1.61)	5.54 ( $\pm$ 0.10)
Lysine	15.8 ( $\pm$ 1.20)	12.8 ( $\pm$ 0.64)	13.2 ( $\pm$ 2.33)
Methionine	0.41 ( $\pm$ 0.29)	0.32 ( $\pm$ 0.37)	0.31 ( $\pm$ 0.07)
Methionine sulfoxide	0.20 ( $\pm$ 0.09)	0.29 ( $\pm$ 0.07)	0.35 ( $\pm$ 0.04)
Methylhistidine	1.26 ( $\pm$ 0.13)	0.92 ( $\pm$ 0.11)	0.83 ( $\pm$ 0.03)
Ornithine	2.54 ( $\pm$ 0.64)	2.37 ( $\pm$ 1.01)	2.79 ( $\pm$ 0.75)
Phenylalanine	1.46 ( $\pm$ 0.08)	1.38 ( $\pm$ 0.06)	1.38 ( $\pm$ 0.01)
Phosphocreatine	7.21 ( $\pm$ 1.64) <sup>a</sup>	5.37 ( $\pm$ 0.61) <sup>a</sup>	17.1 ( $\pm$ 1.41) <sup>b</sup>
Proline	15.8 ( $\pm$ 0.07)	14.7 ( $\pm$ 1.27)	19.0 ( $\pm$ 1.13)
Putrescine	0.05 ( $\pm$ 0.01)	0.05 ( $\pm$ 0.04)	0.02 ( $\pm$ 0.02)
Sarcosine	0.56 ( $\pm$ 0.12)	0.51 ( $\pm$ 0.04)	0.48 ( $\pm$ 0.05)
Serine	21.9 ( $\pm$ 1.13) <sup>b</sup>	18.8 ( $\pm$ 1.27) <sup>a,b</sup>	9.62 ( $\pm$ 1.10) <sup>a</sup>
Serotonin	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)
Spermidine	0.29 ( $\pm$ 0.04)	0.26 ( $\pm$ 0.05)	0.24 ( $\pm$ 0.06)
Spermine	0.07 ( $\pm$ 0.01)	0.07 ( $\pm$ 0.01)	0.07 ( $\pm$ 0.00)
Symmetric	3.82 ( $\pm$ 0.08)	2.80 ( $\pm$ 0.46)	2.73 ( $\pm$ 0.25)

dimethylarginine

Taurine	26.9 (± 2.33)	26.4 (± 1.56)	29.6 (± 1.70)
Threonine	4.88 (± 0.09)	3.90 (± 0.55)	4.08 (± 1.00)
Total dimethylarginine	3.90 (± 0.05)	2.86 (± 0.48)	2.88 (± 0.26)
trans-4-Hydroxyproline	4.21 (± 0.40)	4.03 (± 0.76)	4.53 (± 0.07)
Trimethylamine N-oxide	2.77 (± 0.30)	2.82 (± 0.04)	3.10 (± 0.29)
Tryptophan	0.70 (± 0.06)	0.64 (± 0.11)	0.75 (± 0.02)
Tyramine	0.01 (± 0.00)	0.00*	0.01 (± 0.01)
Tyrosine	0.27 (± 0.04)	0.28 (± 0.16)	0.48 (± 0.18)
Valine	7.00 (± 0.74) <sup>a</sup>	7.92 (± 1.43) <sup>a,b</sup>	10.6 (± 0.07) <sup>b</sup>

**Table S4:** Average concentrations of total metabolites ( $\mu\text{M}$ ) for acid whey powder derived from perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR) feeding systems, determined by LC-MS/MS.

<sup>a,b</sup> indicates values within a row not sharing a common superscript letter differed significantly ( $p < 0.05$ ).

\* denotes where a replicate was below the limit of detection or limit of quantification.

Metabolite ( $\mu\text{M}$ )	GRS	CLV	TMR
Acetylorntine	4.18 ( $\pm$ 0.16)	2.58 ( $\pm$ 0.11)	2.36 ( $\pm$ 1.53)
Alanine	27.7 ( $\pm$ 2.19)	34.1 ( $\pm$ 5.37)	40.6 ( $\pm$ 3.04)
alpha-Aminoadipic acid	23.9 ( $\pm$ 7.14)	28.0 ( $\pm$ 28.61)	40.8 ( $\pm$ 2.26)
Arginine	12.9 ( $\pm$ 1.63)	16.4 ( $\pm$ 3.11)	16.0 ( $\pm$ 1.13)
Asparagine	2.98 ( $\pm$ 0.16)	0.32*	1.32 ( $\pm$ 0.12)
Aspartic acid	6.98 ( $\pm$ 1.03)	13.3 ( $\pm$ 12.47)	4.95 ( $\pm$ 0.34)
Asymmetric dimethylarginine	0.06 ( $\pm$ 0.01)	0.07 ( $\pm$ 0.00)	0.11 ( $\pm$ 0.04)
Betaine	75.9 ( $\pm$ 26.87)	70.7 ( $\pm$ 37.19)	84.9 ( $\pm$ 6.43)
Carnosine	0.33 ( $\pm$ 0.09)	0.30 ( $\pm$ 0.06)	0.39 ( $\pm$ 0.01)
Choline	1160 ( $\pm$ 98.99)	1076 ( $\pm$ 501.34)	950 ( $\pm$ 84.85)
cis-4-Hydroxyproline	0.31 ( $\pm$ 0.00)	0.31*	0.33*
Citrulline	0.33 ( $\pm$ 0.13)	0.66 ( $\pm$ 0.11)	0.86 ( $\pm$ 0.32)
Creatine	483 ( $\pm$ 60.10)	661 ( $\pm$ 119.50)	612 ( $\pm$ 144.96)
Creatinine	99.4 ( $\pm$ 3.68)	104 ( $\pm$ 14.35)	94.0 ( $\pm$ 5.52)
Diacetylspermine	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)
Dihydroxyphenylalanine	0.14 ( $\pm$ 0.01)	0.12 ( $\pm$ 0.01)	0.15 ( $\pm$ 0.05)
Dopamine	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)
Glutamic acid	262 ( $\pm$ 37.48)	255 ( $\pm$ 69.30)	234 ( $\pm$ 25.46)
Glutamine	2.39 ( $\pm$ 1.74) <sup>a</sup>	2.97 ( $\pm$ 1.07) <sup>a</sup>	12.3 ( $\pm$ 0.42) <sup>b</sup>
Glycine	56.1 ( $\pm$ 9.62)	57.3 ( $\pm$ 0.28)	41.2 ( $\pm$ 0.49)
Histidine	2.34 ( $\pm$ 0.55)	2.49 ( $\pm$ 0.01)	3.21 ( $\pm$ 0.15)
Isoleucine	2.80 ( $\pm$ 0.02)	2.58 ( $\pm$ 1.53)	3.73 ( $\pm$ 1.13)
Leucine	12.0 ( $\pm$ 5.82)	8.28 ( $\pm$ 3.57)	14.6 ( $\pm$ 0.71)
Lysine	12.7 ( $\pm$ 4.04)	12.5 ( $\pm$ 1.13)	11.4 ( $\pm$ 7.95)
Methionine	0.47 ( $\pm$ 0.10)	0.49 ( $\pm$ 0.10)	0.43 ( $\pm$ 0.24)
Methionine sulfoxide	0.16 ( $\pm$ 0.21)	0.44 ( $\pm$ 0.25)	0.14 ( $\pm$ 0.08)
Methylhistidine	1.27 ( $\pm$ 0.14)	1.36 ( $\pm$ 0.13)	0.97 ( $\pm$ 0.16)
Ornithine	3.22 ( $\pm$ 0.83)	2.68 ( $\pm$ 0.04)	3.35 ( $\pm$ 0.82)
Phenylalanine	1.22 ( $\pm$ 0.09)	1.09 ( $\pm$ 0.21)	1.41 ( $\pm$ 0.29)
Phosphocreatine	3.56 ( $\pm$ 0.24)	3.66 ( $\pm$ 0.84)	4.35 ( $\pm$ 0.47)
Proline	14.8 ( $\pm$ 2.47)	14.7 ( $\pm$ 2.12)	19.1 ( $\pm$ 2.26)
Putrescine	0.08 ( $\pm$ 0.02)	0.05*	0.01*
Sarcosine	0.41 ( $\pm$ 0.06)	0.39 ( $\pm$ 0.01)	0.40 ( $\pm$ 0.01)
Serine	20.2 ( $\pm$ 0.64)	17.5 ( $\pm$ 8.27)	11.2 ( $\pm$ 2.63)
Serotonin	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)
Spermidine	0.71 ( $\pm$ 0.04)	0.58 ( $\pm$ 0.15)	0.56 ( $\pm$ 0.12)
Spermine	0.26 ( $\pm$ 0.07)	0.22 ( $\pm$ 0.08)	0.30 ( $\pm$ 0.21)
Symmetric	3.40 ( $\pm$ 0.38)	2.94 ( $\pm$ 1.58)	2.77 ( $\pm$ 0.03)

dimethylarginine			
Taurine	26.0 (± 5.16)	26.0 (± 3.04)	30.0 (± 3.46)
Threonine	1.69 (± 0.76)	1.02 (± 0.40)	1.28 (± 0.41)
Total dimethylarginine	3.46 (± 0.37)	3.01 (± 1.58)	2.88 (± 0.07)
trans-4-Hydroxyproline	4.09 (± 1.12)	4.01 (± 0.11)	4.48 (± 0.38)
Trimethylamine N-oxide	2.57 (± 0.05)	3.21 (± 0.69)	3.13 (± 0.48)
Tryptophan	0.45 (± 0.11)	0.57 (± 0.10)	0.58 (± 0.12)
Tyramine	0.01 (± 0.00)	0.00 (± 0.00)	0.00*
Tyrosine	0.00*	0.00*	0.08*
Valine	6.80 (± 1.97) <sup>a</sup>	7.29 (± 0.69) <sup>a</sup>	12.0 (± 1.63) <sup>b</sup>

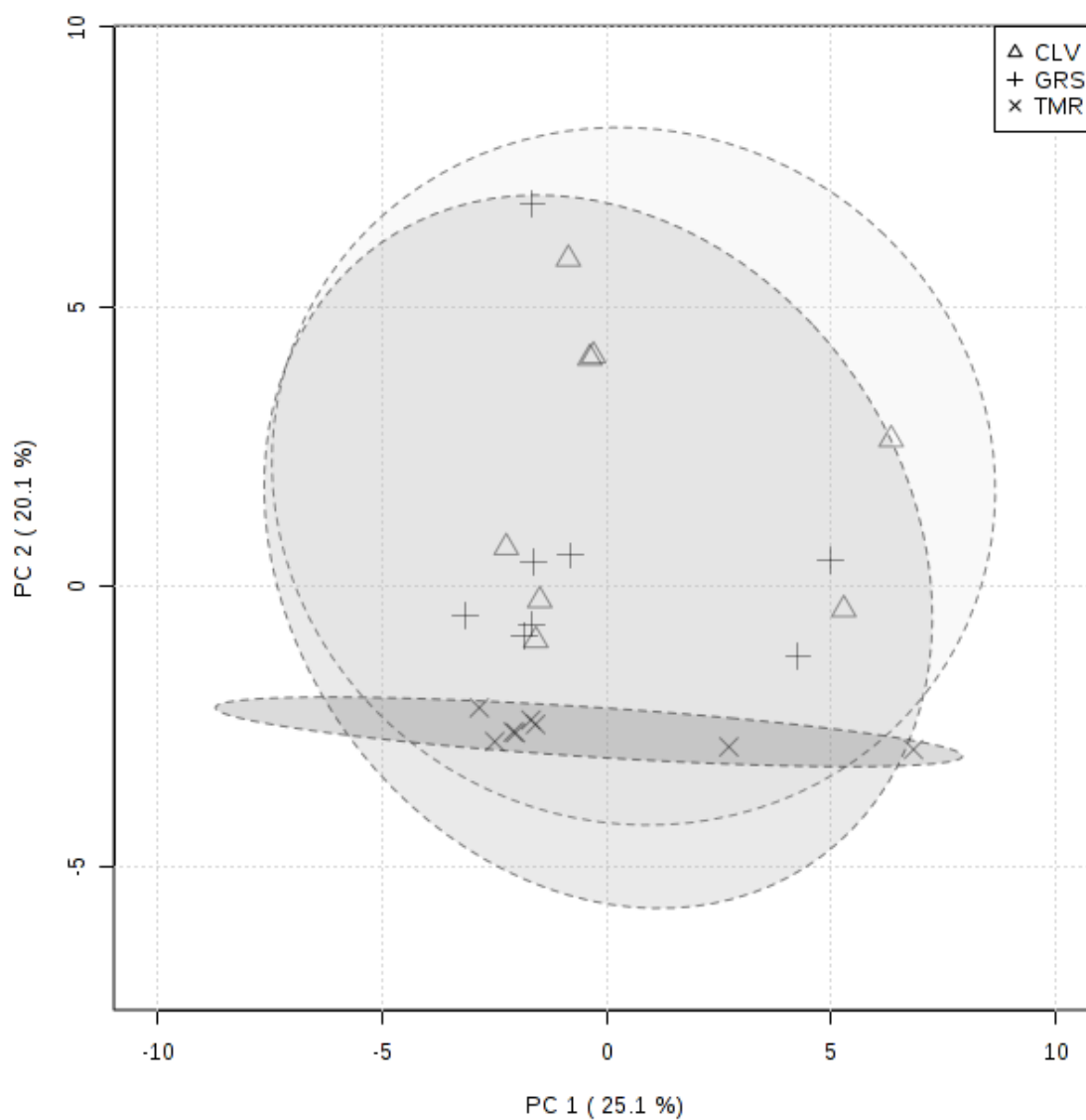


**Table S5:** Average concentrations of total metabolites ( $\mu\text{M}$ ) from skim milk powder, sweet whey powder, ideal whey powder and acid whey powder derived from perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR) feeding systems, determined by LC-MS/MS.

<sup>a,b</sup> indicates values within a row not sharing a common superscript letter differed significantly ( $p < 0.05$ ).

Metabolite ( $\mu\text{M}$ )	GRS	CLV	TMR
Acetylorntine	1.84 ( $\pm$ 1.57)	1.26 ( $\pm$ 0.90)	1.20 ( $\pm$ 0.77)
Alanine	35.8 ( $\pm$ 7.02) <sup>a,b</sup>	33.1 ( $\pm$ 2.15) <sup>a</sup>	41.0 ( $\pm$ 1.40) <sup>b</sup>
alpha-Aminoadipic acid	12.2 ( $\pm$ 7.93)	12.2 ( $\pm$ 10.66)	15.5 ( $\pm$ 16.90)
Arginine	14.1 ( $\pm$ 1.36)	14.9 ( $\pm$ 1.11)	15.8 ( $\pm$ 0.74)
Asparagine	1.77 ( $\pm$ 0.81) <sup>a,b</sup>	1.12 ( $\pm$ 0.92) <sup>a</sup>	2.26 ( $\pm$ 1.10) <sup>b</sup>
Aspartic acid	9.83 ( $\pm$ 1.99)	15.0 ( $\pm$ 3.62)	10.5 ( $\pm$ 4.22)
Asymmetric dimethylarginine	0.10 ( $\pm$ 0.05)	0.06 ( $\pm$ 0.01)	0.10 ( $\pm$ 0.04)
Betaine	79.1 ( $\pm$ 5.10) <sup>a,b</sup>	65.6 ( $\pm$ 4.16) <sup>a</sup>	83.9 ( $\pm$ 8.66) <sup>b</sup>
Carnosine	0.36 ( $\pm$ 0.03) <sup>a</sup>	0.36 ( $\pm$ 0.05) <sup>a</sup>	0.44 ( $\pm$ 0.04) <sup>b</sup>
Choline	1261 ( $\pm$ 108.73) <sup>b</sup>	1085 ( $\pm$ 95.49) <sup>a,b</sup>	945 ( $\pm$ 64.43) <sup>a</sup>
cis-4-Hydroxyproline	0.32 ( $\pm$ 0.01)	0.32 ( $\pm$ 0.01)	0.32 ( $\pm$ 0.01)
Citrulline	1.28 ( $\pm$ 0.67) <sup>a</sup>	1.35 ( $\pm$ 0.49) <sup>a</sup>	2.07 ( $\pm$ 0.82) <sup>b</sup>
Creatine	567 ( $\pm$ 130.27)	557 ( $\pm$ 70.49)	559 ( $\pm$ 46.08)
Creatinine	112 ( $\pm$ 10.36) <sup>b</sup>	99.3 ( $\pm$ 6.97) <sup>a,b</sup>	90.1 ( $\pm$ 11.96) <sup>a</sup>
Diacetylspermine	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)	0.03 ( $\pm$ 0.00)
Dihydroxyphenylalanine	0.18 ( $\pm$ 0.03)	0.16 ( $\pm$ 0.03)	0.16 ( $\pm$ 0.01)
Dopamine	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)	0.08 ( $\pm$ 0.00)
Glutamic acid	275 ( $\pm$ 28.76)	276 ( $\pm$ 18.09)	248 ( $\pm$ 22.43)
Glutamine	3.04 ( $\pm$ 1.42) <sup>a</sup>	2.82 ( $\pm$ 1.97) <sup>a</sup>	10.8 ( $\pm$ 2.56) <sup>b</sup>
Glycine	70.4 ( $\pm$ 16.30)	58.6 ( $\pm$ 3.49)	54.9 ( $\pm$ 14.26)
Histidine	2.41 ( $\pm$ 0.13) <sup>a</sup>	2.44 ( $\pm$ 0.07) <sup>a</sup>	3.18 ( $\pm$ 0.17) <sup>b</sup>
Isoleucine	2.78 ( $\pm$ 0.25) <sup>a</sup>	2.56 ( $\pm$ 0.16) <sup>a</sup>	3.93 ( $\pm$ 0.27) <sup>b</sup>
Leucine	5.10 ( $\pm$ 4.60) <sup>a,b</sup>	4.32 ( $\pm$ 2.65) <sup>a</sup>	7.47 ( $\pm$ 4.80) <sup>b</sup>
Lysine	15.7 ( $\pm$ 2.37)	13.3 ( $\pm$ 0.74)	12.5 ( $\pm$ 0.78)
Methionine	0.72 ( $\pm$ 0.42)	0.69 ( $\pm$ 0.40)	0.53 ( $\pm$ 0.29)
Methionine sulfoxide	0.21 ( $\pm$ 0.15)	0.40 ( $\pm$ 0.10)	0.24 ( $\pm$ 0.09)
Methylhistidine	1.25 ( $\pm$ 0.03) <sup>b</sup>	1.10 ( $\pm$ 0.19) <sup>a,b</sup>	0.93 ( $\pm$ 0.07) <sup>a</sup>
Ornithine	2.72 ( $\pm$ 0.42) <sup>a,b</sup>	2.27 ( $\pm$ 0.33) <sup>a</sup>	3.26 ( $\pm$ 0.65) <sup>b</sup>
Phenylalanine	1.46 ( $\pm$ 0.20) <sup>b</sup>	1.29 ( $\pm$ 0.19) <sup>a</sup>	1.41 ( $\pm$ 0.03) <sup>a,b</sup>
Phosphocreatine	7.12 ( $\pm$ 2.58) <sup>a</sup>	5.46 ( $\pm$ 1.30) <sup>a</sup>	15.1 ( $\pm$ 7.74) <sup>b</sup>
Proline	15.6 ( $\pm$ 0.85) <sup>a</sup>	14.7 ( $\pm$ 0.61) <sup>a</sup>	18.9 ( $\pm$ 0.41) <sup>b</sup>
Putrescine	0.06 ( $\pm$ 0.01) <sup>b</sup>	0.06 ( $\pm$ 0.02) <sup>b</sup>	0.03 ( $\pm$ 0.02) <sup>a</sup>
Sarcosine	0.50 ( $\pm$ 0.07)	0.48 ( $\pm$ 0.07)	0.41 ( $\pm$ 0.06)
Serine	22.4 ( $\pm$ 2.15) <sup>c</sup>	18.3 ( $\pm$ 0.60) <sup>b</sup>	10.0 ( $\pm$ 0.85) <sup>a</sup>
Serotonin	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)
Spermidine	0.54 ( $\pm$ 0.27)	0.49 ( $\pm$ 0.23)	0.48 ( $\pm$ 0.27)
Spermine	0.21 ( $\pm$ 0.20)	0.21 ( $\pm$ 0.21)	0.26 ( $\pm$ 0.24)
Symmetric	4.04 ( $\pm$ 0.54) <sup>b</sup>	3.08 ( $\pm$ 0.25) <sup>a</sup>	2.98 ( $\pm$ 0.30) <sup>a</sup>

dimethylarginine			
Taurine	27.3 ( $\pm$ 1.72) <sup>a,b</sup>	26.4 ( $\pm$ 1.41) <sup>a</sup>	30.1 ( $\pm$ 0.38) <sup>b</sup>
Threonine	4.06 ( $\pm$ 1.61) <sup>b</sup>	3.03 ( $\pm$ 1.36) <sup>a</sup>	3.69 ( $\pm$ 1.65) <sup>a,b</sup>
Total dimethylarginine	4.15 ( $\pm$ 0.59) <sup>b</sup>	3.13 ( $\pm$ 0.24) <sup>a</sup>	3.09 ( $\pm$ 0.28) <sup>a</sup>
trans-4-Hydroxyproline	4.38 ( $\pm$ 0.33)	4.18 ( $\pm$ 0.21)	4.49 ( $\pm$ 0.06)
Trimethylamine N-oxide	3.00 ( $\pm$ 0.40)	3.01 ( $\pm$ 0.22)	3.19 ( $\pm$ 0.10)
Tryptophan	0.72 ( $\pm$ 0.19)	0.68 ( $\pm$ 0.10)	0.71 ( $\pm$ 0.10)
Tyramine	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)	0.01 ( $\pm$ 0.00)
Tyrosine	0.33 ( $\pm$ 0.08) <sup>a,b</sup>	0.28 ( $\pm$ 0.00) <sup>a</sup>	0.43 ( $\pm$ 0.24) <sup>b</sup>
Valine	7.45 ( $\pm$ 0.76) <sup>a</sup>	7.56 ( $\pm$ 0.34) <sup>a</sup>	11.09 ( $\pm$ 0.66) <sup>b</sup>



**Figure S1.** Principal component analysis (PCA) score plot for metabolomics analysis of protein ingredients from perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR) feeding systems, determined by LC-MS/MS.