Glutamine and glucose metabolism in suckling low birthweight piglets supplemented with glutamine

Daria De Leonardis (ESR5)

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

Abstract

Glutamine and glucose metabolism in suckling low birth weight piglets supplemented with glutamine

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Glutamine (Gln) supplementation has been shown to be beneficial in growing piglets. To study the effect of glutamine supplementation on metabolic pathways in suckling piglets, male German Landrace piglets with low (L; 0.8-1.2 kg) and normal birthweight (N; 1.5-1.9 kg) were selected. At 24 h after birth, 10 L and N piglets/group were allocated to daily Gln (1 g/kg BW/d; L-Gln, N-Gln) or water (W, 6 ml; L-W, N-W) supplementation. At age 14 d, piglets received orally Gln (0.33 g/kg BW) plus 13C5 Gln (10 mg/kg BW), and at 16 d, glucose (Glc; 0.4 g/kg BW) plus 13C6 Glc (10 mg/kg BW). Blood was collected before (-15 min, basal) and half-hourly until 300 min after tracer administration via a jugular catheter. Mass spectrometry was used to measure red blood cell 13CO2 enrichment (E) derived from oxidation of 13C5 Gln and 13C6 Glc, plasma 13C5 Gln E, and plasma 13C3 Glc E, newly synthetized from 13C5 Gln tracer carbon. Area under the 13C enrichment-time-curve (AUC), maximum enrichment (Emax) and time to maximum enrichment (Tmax) were computed by curve fitting. Statistical evaluation was performed by Student t-tests. Preliminary results show that 13CO2 E Tmax from 13C5 Gln and 13C6 Glc oxidation was greater in N-Gln than in L-Gln and N-W (P<0.05). Plasma 13C5 Gln E AUC tended to be lower (141.4 vs 174.4 mole % excess (MPE)*min; P=0.1) and Emax was lower (0.95 vs 1.24 MPE; P<0.05) in N-Gln than in N-W. Plasma 13C3 Glc E AUC derived from 13C5 Gln metabolism tended to be greater in L-Gln than in L-W and N-Gln piglets (14.9 vs 8.5 vs 9.2 MPE*min; P=0.1). The 13C3 Glc Tmax was greater in N-Gln than in L-Gln and N-W groups (91.1 vs 57.9 vs 55.9 min; n=3/group; P<0.05). Our data suggest that L piglets supplemented with Gln oxidized Gln and Glc faster than N piglets. This agrees with greater utilization of glutamine carbon for Glc de novo synthesis in L-Gln piglets. The lower plasma 13C5 Gln E in N-Gln piglets might indicate a greater dilution by endogenous Gln production. These results must be confirmed by further investigations of Gln and Glc metabolism.

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Pig production challenges

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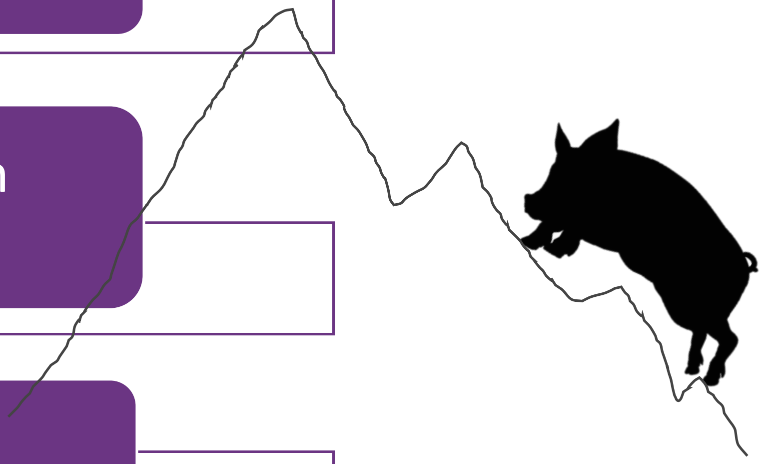
- Ethical and welfare issues*
- Low public acceptance of current farming practices[ł]
- Large litters: increased numbers of intrauterine growth restricted and low birthweight piglets[ł]
- High pre-weaning mortality rate: 1/5 piglets[ł]
- Inefficient use of resources and loss in profit: ~ 23 €/litter[§]



*Albernaz-Gonçalves et al, 2021
[ł] Farmers and Edwards, 2022
[§] Stygar et al, 2022

Picture: pixabay.com

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Low birthweight piglets

15–20% of all piglets born: **< 1 kg birthweight**

40% mortality vs 15% mortality in normal birthweight piglets

Higher mortality:

- **Lower** energy reserves
- **Delayed** access to colostrum
- **Reduced** intestinal size and maturity
- **Impaired** gut barrier function
- **Lower** postnatal growth

Elliot and Lodge, 1997

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Nutritional strategies for increasing vitality in suckling piglets

Picture: www.alamy.com

Oral supplementation

- Milk replacer
- Probiotics and prebiotics
- Colostrum
- Fecal microbiota transplant
- Glutamine

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Blavi et al, 2021

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Why glutamine?

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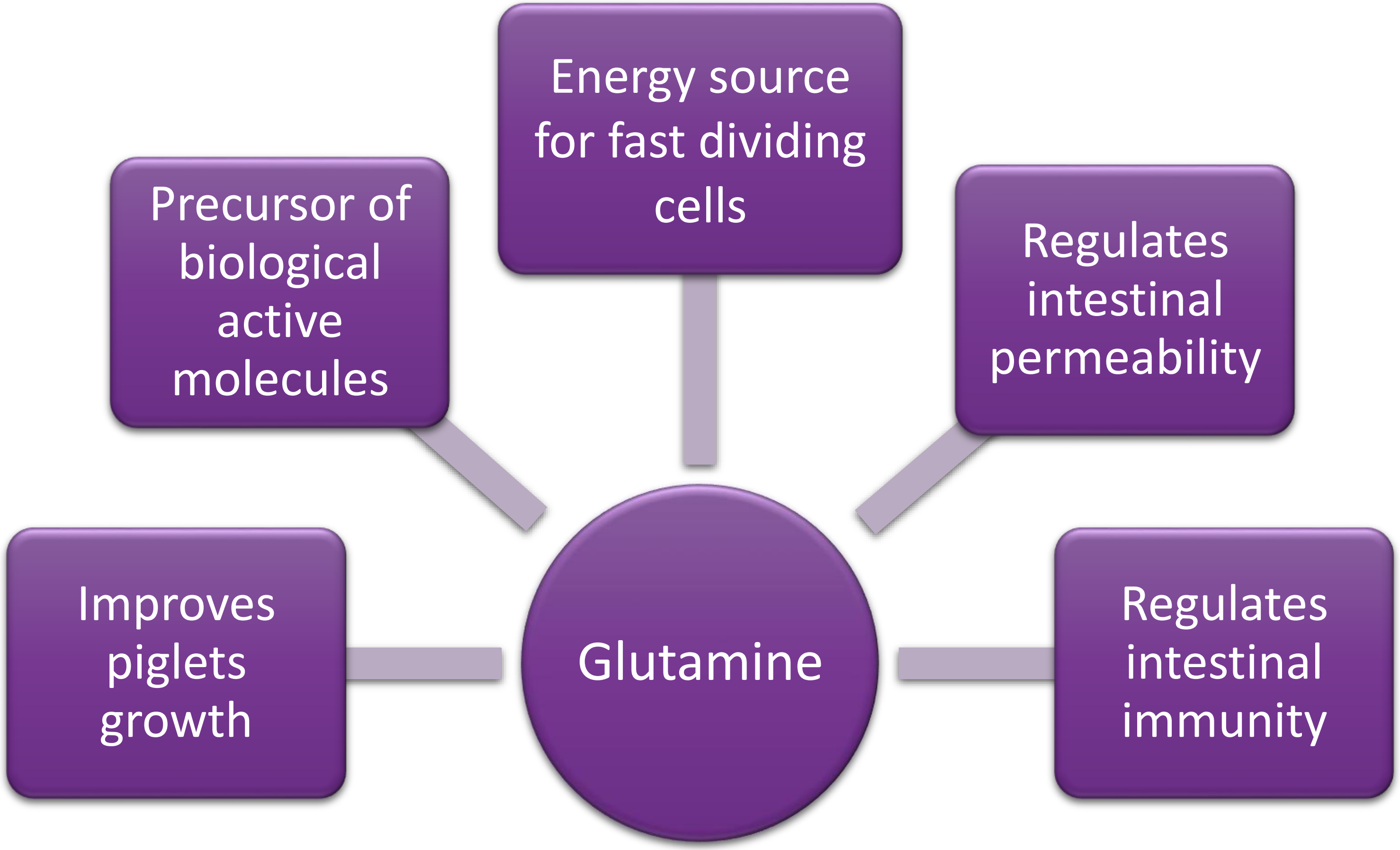

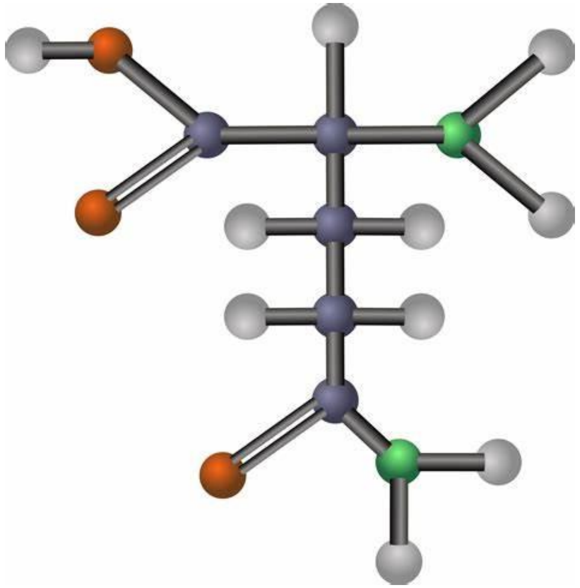

Glutamine (openclipart.org)

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Wu et al, 2011

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Glutamine supplementation

Research questions

Does glutamine supplementation improve suckling low birthweight piglet growth?

Does glutamine supplementation affect glutamine and glucose metabolism?

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Experimental set up

Piglet selection criteria

- Pure German Landrace piglets, sourced from the FBN experimental pig facility
- Born from **parity 2 to 9**
- Standardized litter size **14 piglets,** 24 hours after farrowing
- **Only male low and normal birthweight littermates**

Low birthweight (L)
(0.8-1.2 kg)
→ **L-Gln** | **L-Water**

Normal birthweight (N)
(1.5-1.9 kg)
→ **N-Gln** | **N-Water**

Supplementation

Daily: 1 g Gln/kg BW in water or equal volume of water (Control)

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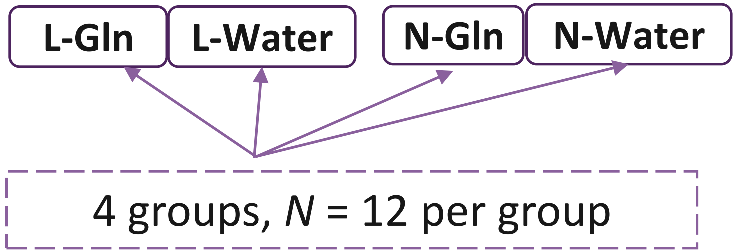
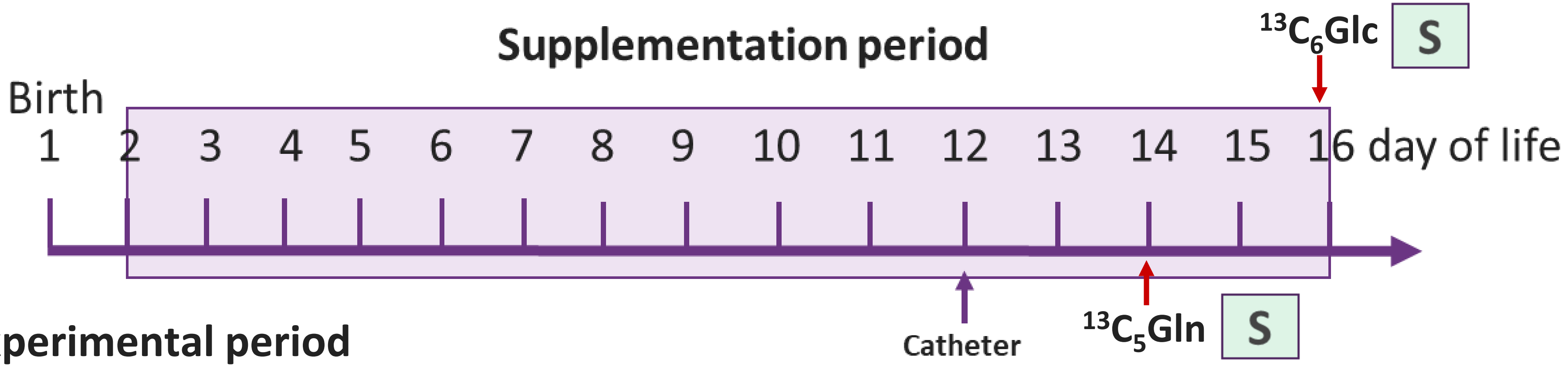
Experiment

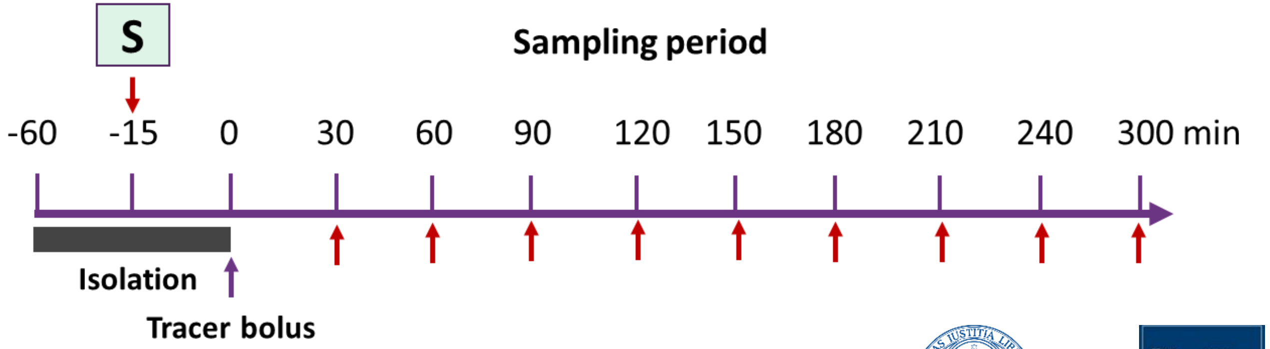
Supplementation protocol
- Oral, 3x daily (7h, 12h and 17h)
- Gln or water (control)

Bodyweight daily





Experimental period
- 12 d, **Jugular catheter insertion**
- 14 d, **Glutamine metabolism**; $^{13}C_5$Gln-Gln
- 16 d, **Glucose metabolism;** $^{13}C_6$Glc-Glc



Samples: Red blood cells and plasma **S**

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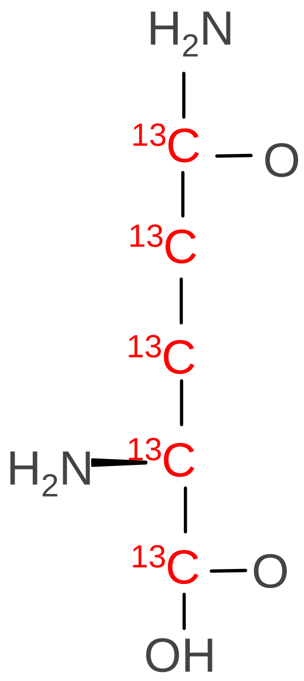
Stable isotope tracers

14 d

L-$^{13}C_5$-Glutamine

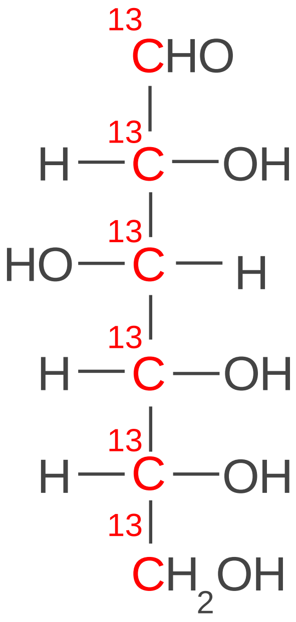
M+5



16 d

D-$^{13}C_6$-Glucose

M+6



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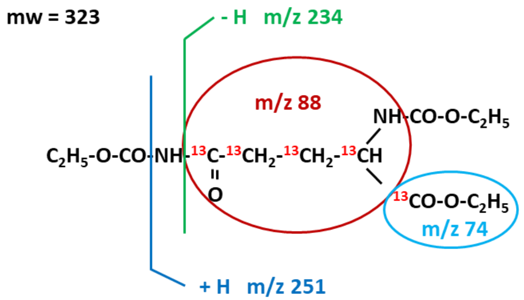
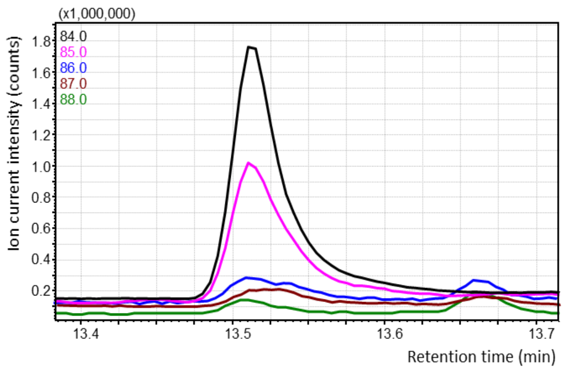
Methods - Mass Spectrometry

Gas Chromatography Mass Spectrometry (GC/MS)

Plasma

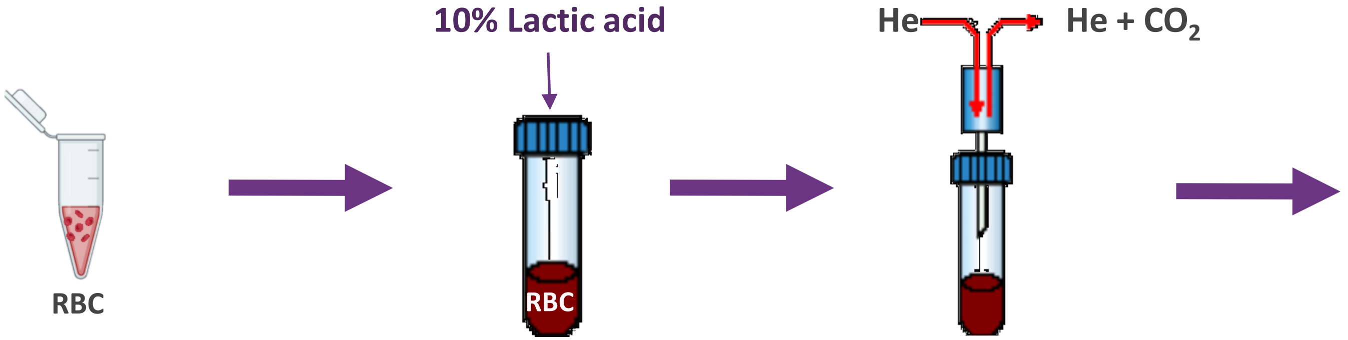


$^{13}C_5$-Glutamine-di-N-ethoxycarbonyl ethyl ester: fragments



1. Derivatization
2. Injection of the derivative
3. $^{13}C_5$ glutamine enrichment measurement

Isotope Ratio Mass Spectrometry (IRMS)



to IRMS = $^{13}CO_2/^{12}CO_2$

1. Isolation of CO_2
2. Injection of He = outflow of He+CO_2
3. $^{13}CO_2$ enrichment measurement

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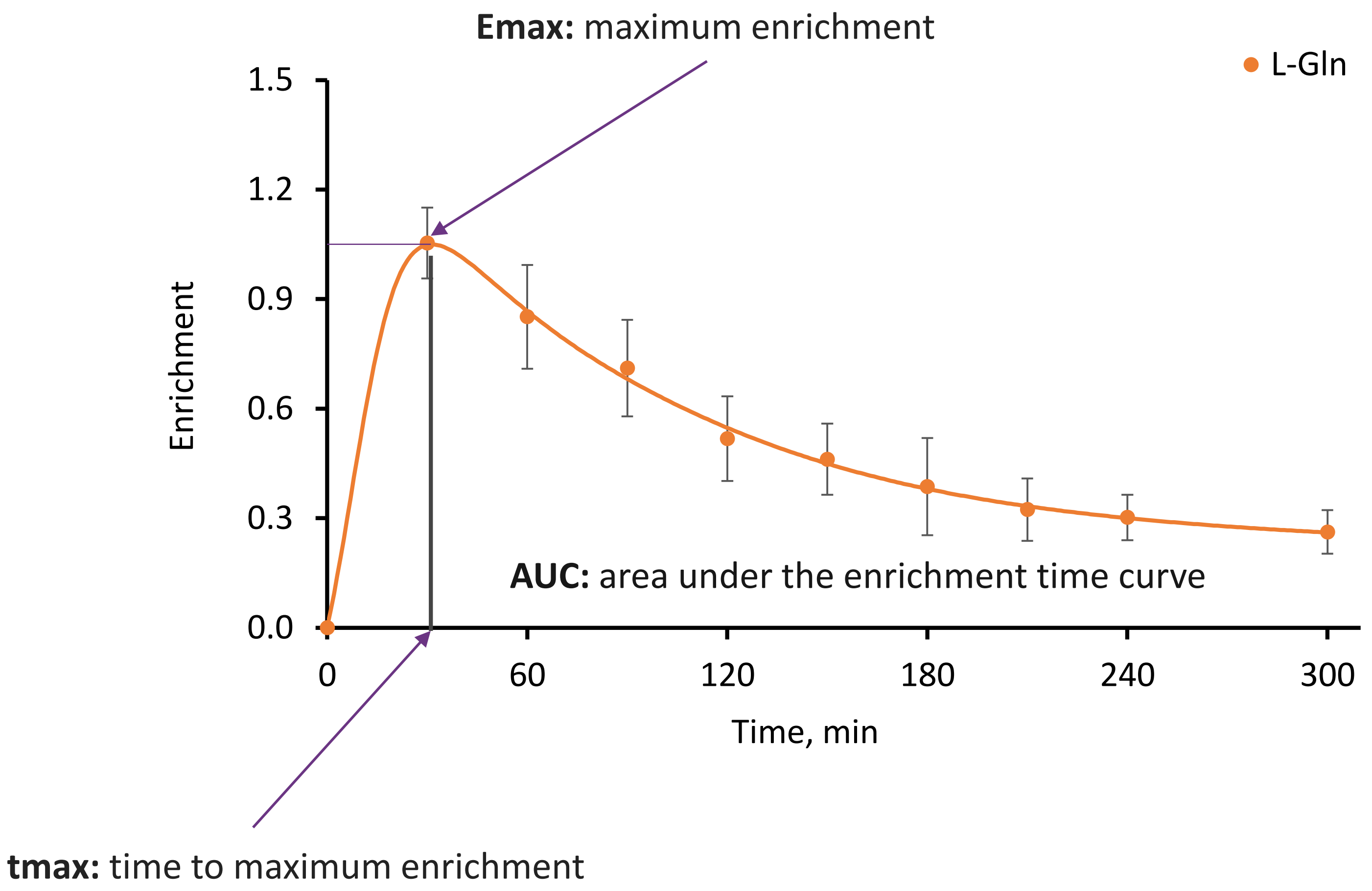
Methods – Tracer dilution paramenters



E: enrichment, mean ± SEM

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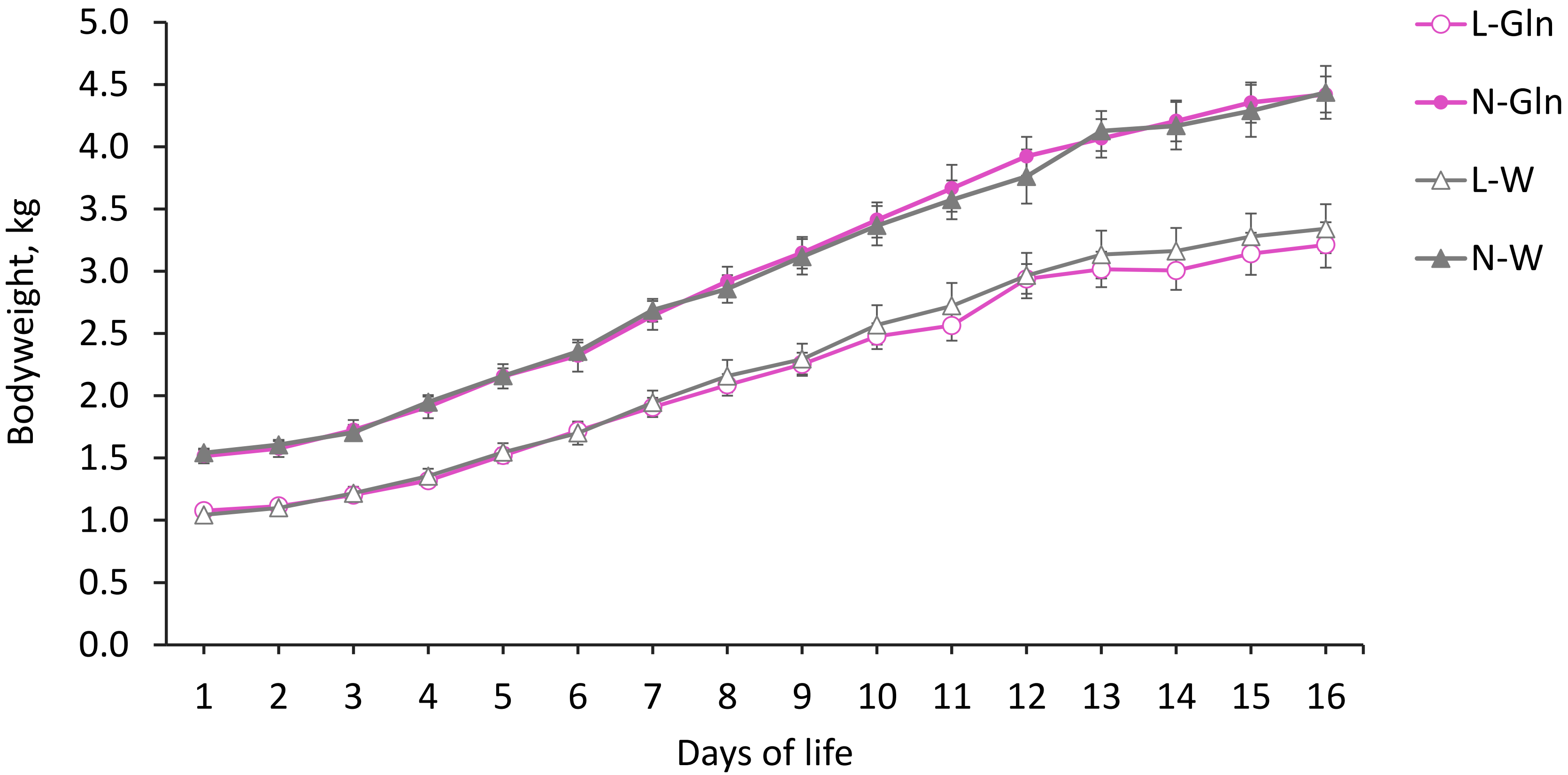
Preliminary results -
Bodyweight data



L/N-Gln, n = 12 per group
L/N-W, n = 11 per group
Means ± SEM

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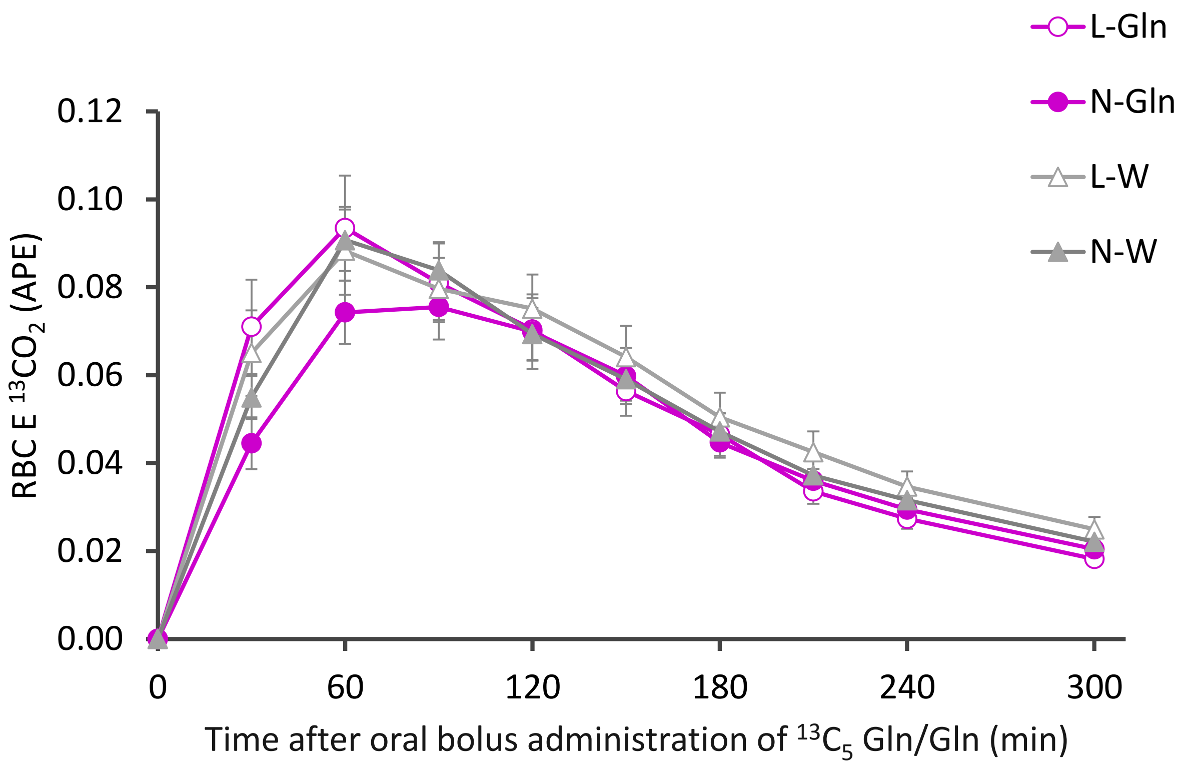
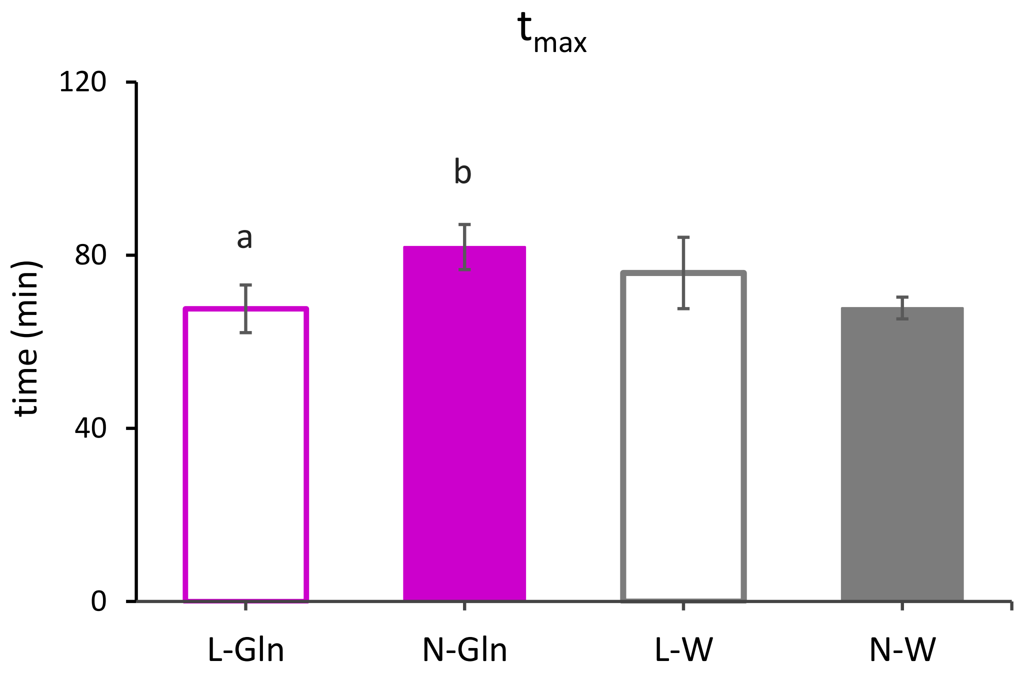
Red blood cell (RBC) $^{13}CO_2$ enrichment derived from $^{13}C_5$ glutamine





L/N-Gln, n = 12 per group
L/N-W, n = 11 per group
Means ± SEM
[a,b]Different from NBW piglets within Supplementation group ($P < 0.05$).
tmax: time to maximum enrichment

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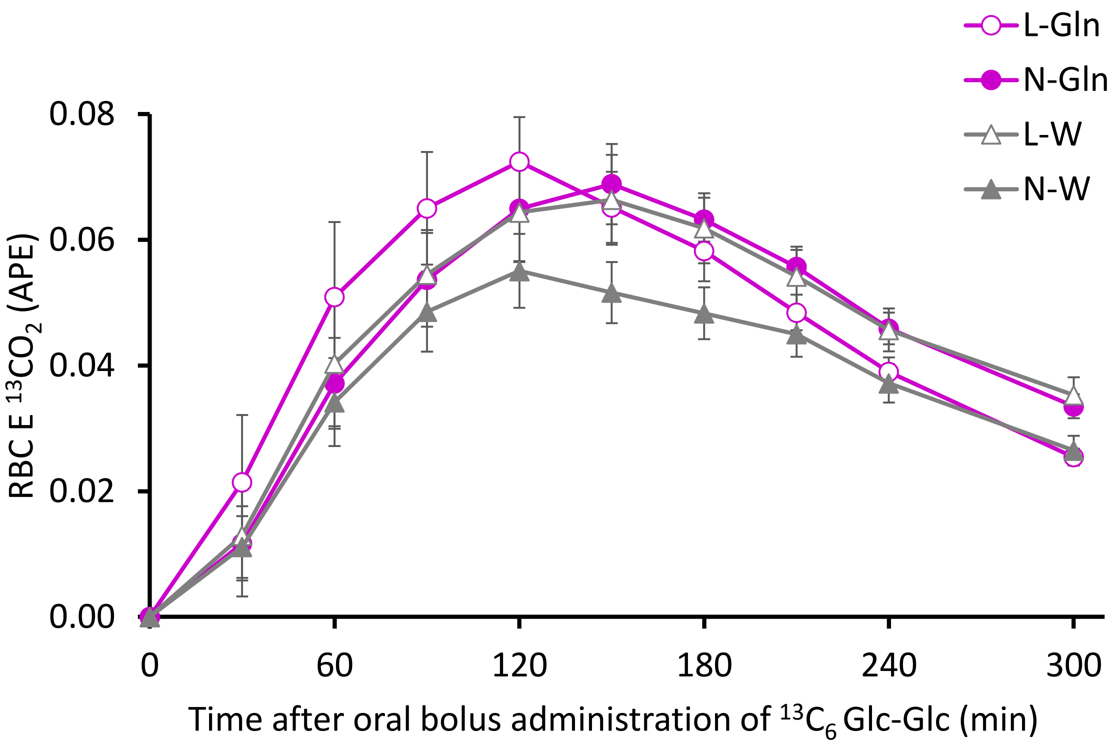
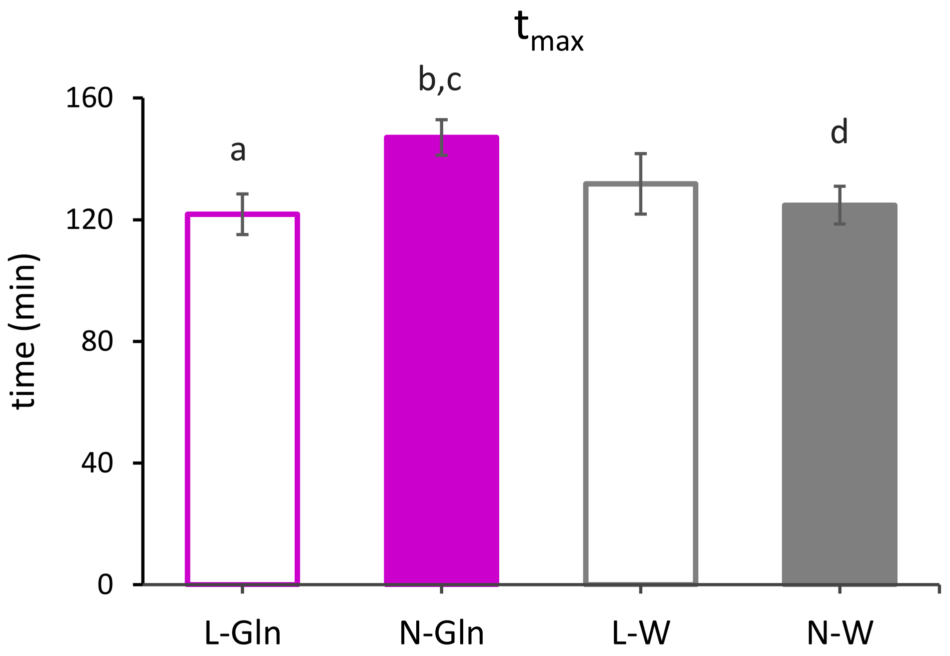
Red blood cell (RBC) $^{13}CO_2$ enrichment derived from $^{13}C_6$ glucose





L/N-Gln, n = 12 per group
L/N-W, n = 11 per group
Means ± SEM
[a,b]Different from NBW piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water supplemented piglets within birthweight group ($P < 0.05$).
tmax: time to maximum enrichment

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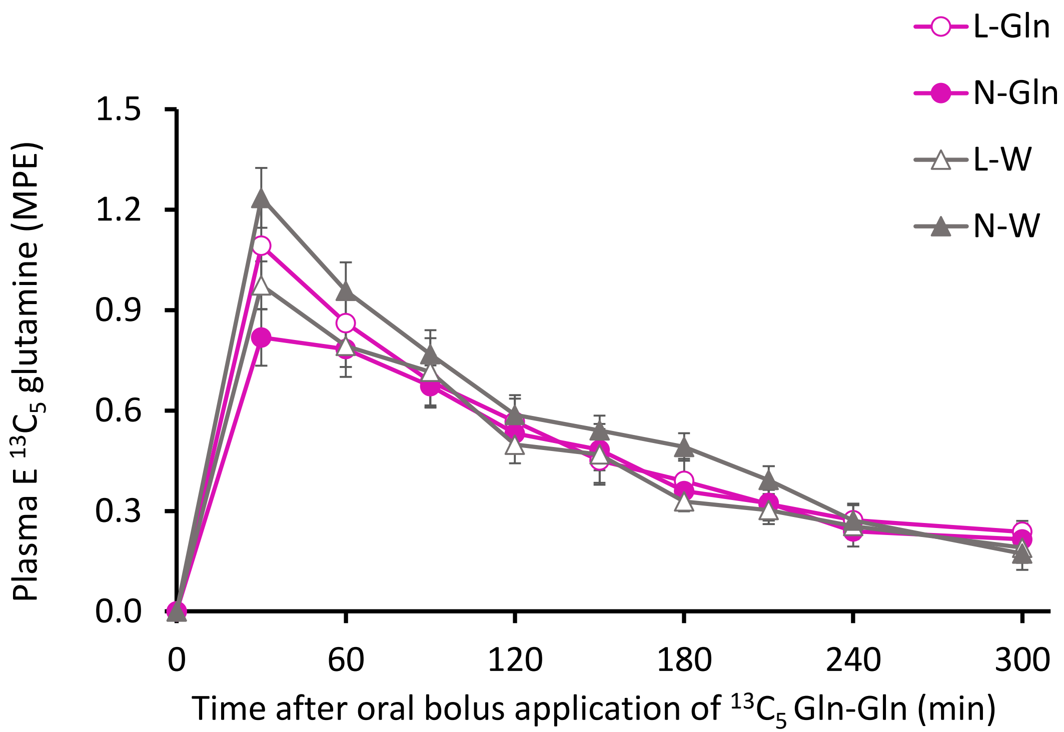
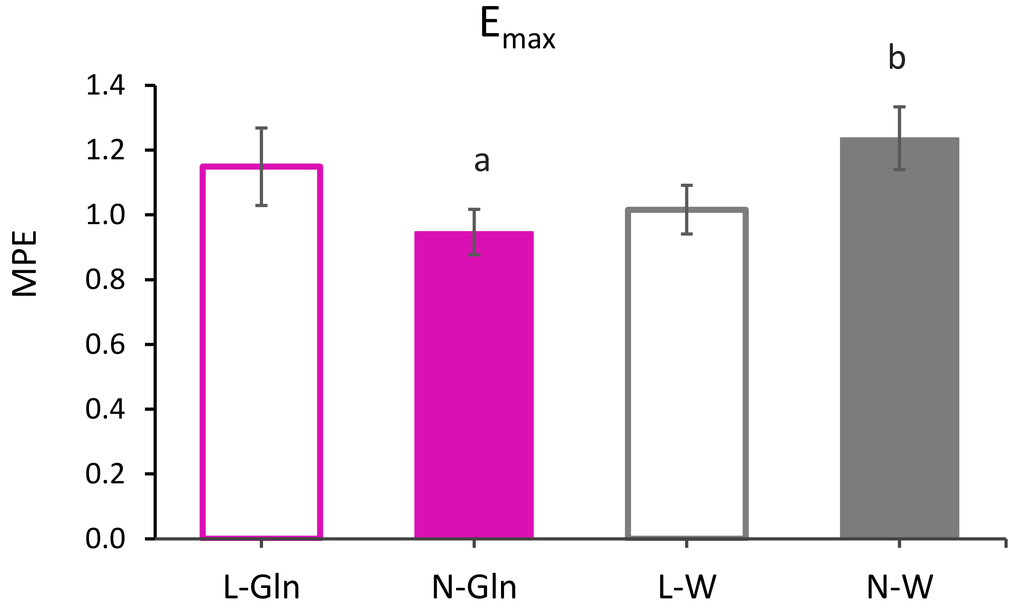
Plasma enrichment of $^{13}C_5$ glutamine





L/N-Gln, n = 12 per group
L/N-W, n = 11 per group
Means ± SEM
[a,b]Different from Water supplemented piglets within Birth weight group ($P < 0.05$).
Emax: maximum enrichment

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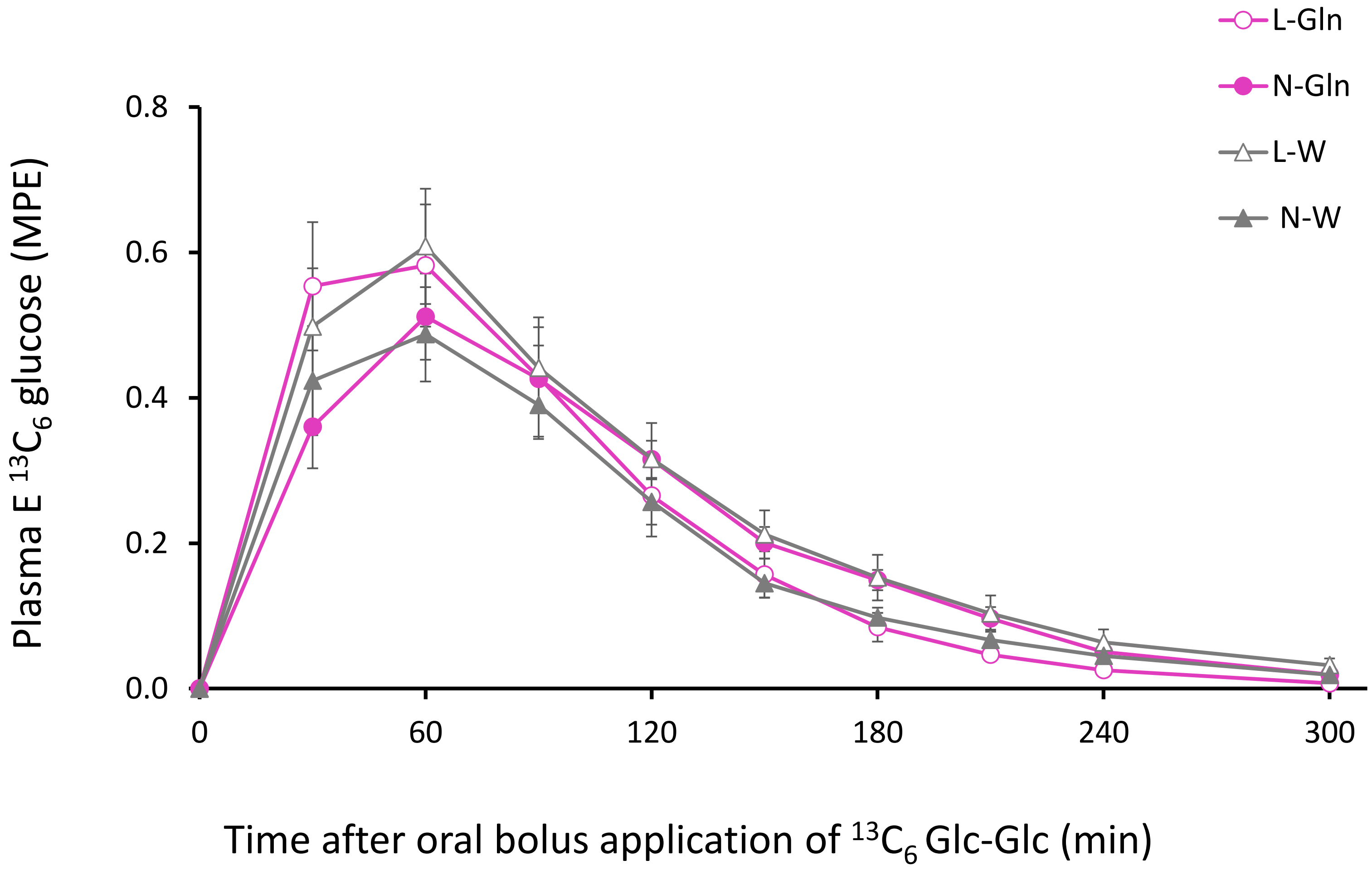
Plasma enrichment of $^{13}C_6$ glucose



L/N-Gln, n = 12 per group
L/N-W, n = 11 per group
Means ± SEM

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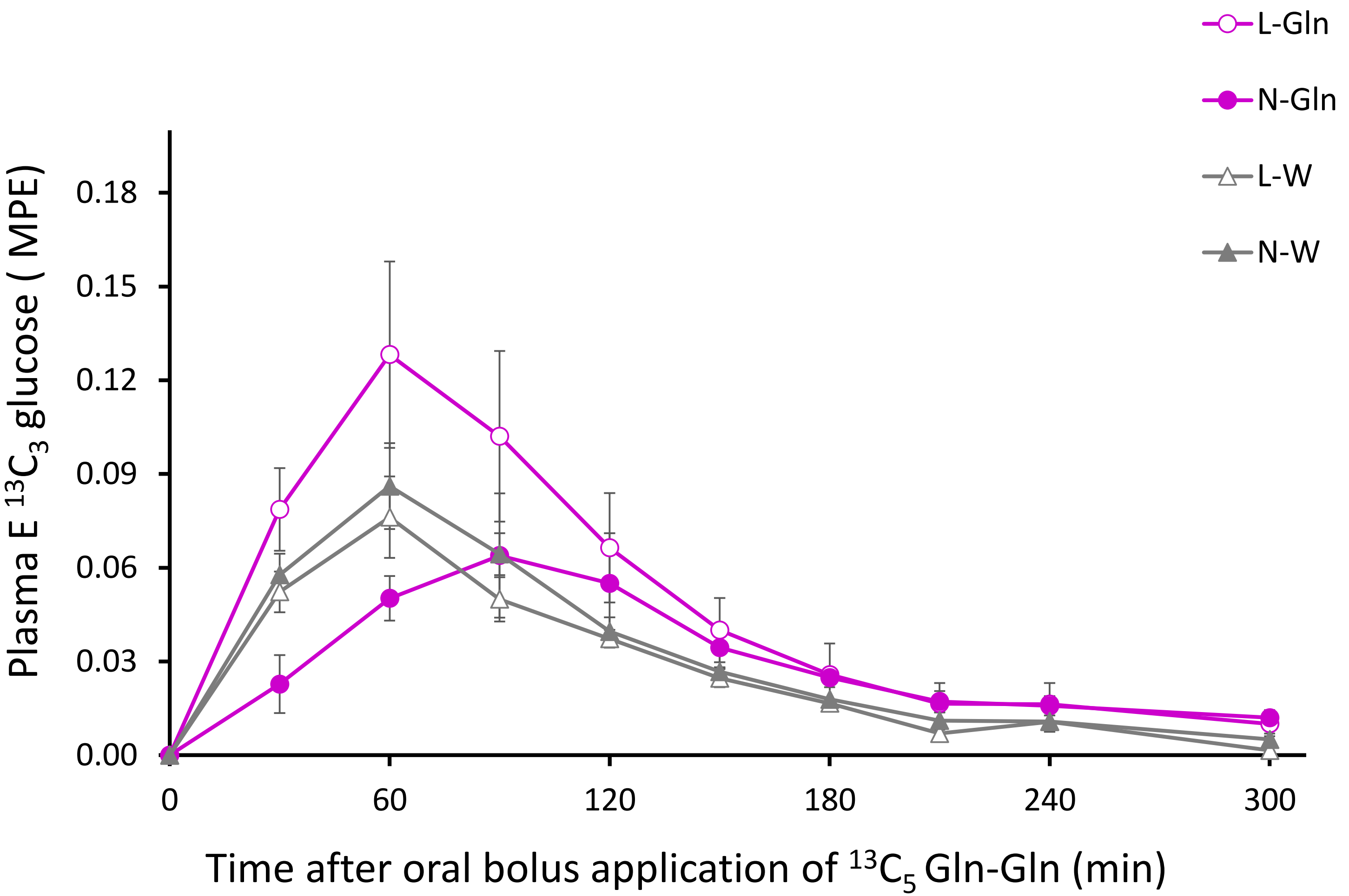
Plasma enrichment of $^{13}C_3$-glucose derived from $^{13}C_5$-glutamine



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Glutamine

Glutamate

α-Ketoglutarate

Krebs cycle

Oxalacetate

Phosphoenolpyruvate

Glucose

L/N-Gln, n = 3 per group
L/N-W, n = 3 per group
Means ± SEM

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Take home messages

What are the effects of glutamine supplementation on low birthweight piglet growth?

- Gln did not improve L piglets bodyweight

What are the effects on glutamine and glucose metabolism?

$^{13}CO_2$

- L-Gln piglets oxidise faster Gln and Glc than N piglets
- L-Gln piglets have a greater utilization of Gln carbon for Glc de novo synthesis

Plasma glutamine enrichment

- N-Gln piglets have lower plasma $^{13}C_5$ Gln enrichment N-W piglets.
- N-Gln have greater metabolism in the splanchnic tissue when tissues are adapted to greater Gln supply.

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THANK YOU

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