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Optimal gut function in monogastric livestock

Glutamine supplementation during the suckling period and its influence on piglet growth and intestinal metabolism

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Challenges of modern pig production.

Environmental impact (NH_3, N_2O, CO_2, CH_4 direct and indirect (50 – 70%).

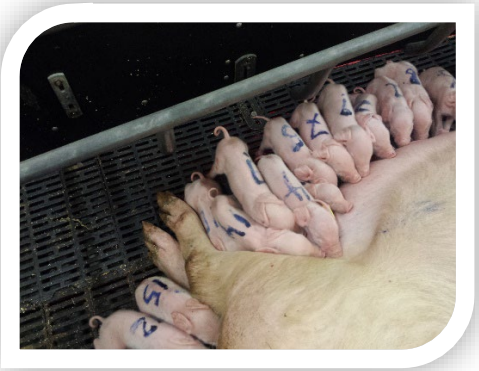
Animal welfare (farrowing crates, lack of space).[2]

Negative public perception of mass production pig farming (European Livestock Voice).[1]

Increased production costs (feed, energy, staff).

Loss of breed diversity (95% of modern production is just a few breeds) – bred for productivity.[1]

Increased litter sizes.



- ✓ Has decreased the number of litters required to produce 1000 weaned piglets from **126** in 2009 to **80** in 2021[2]
- ❖ Has led to increased numbers of low birthweight piglets, which have a…[2]
- ❖ …Higher pre-weaning mortality[2]

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Low birthweight piglets – definition and problems.

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Definition: There is no commonly agreed definition for what constitutes a low birthweight piglet. Researchers have used birthweight cutoffs or individual / group litter birthweight variation as methods to define and identify low birthweight piglets in their studies.

- Slower growth:[5,6] ✓
  - a. Impaired muscle development and subsequent lower carcass quality.
- Altered metabolism:[7,8]
  - a. Impaired pre-weaning glucose tolerance – led to the identification of ***myo*-inositol** as a biomarker of low birthweight in pigs
- Impaired gastrointestinal development:[9,10] ✓
  - a. Impaired pre-weaning intestinal structure and lower enzymatic activity
  - b. Dysfunctional intestinal mitochondrial function and antioxidative defense at weaning.

✓ The amino acid Glutamine has been shown to improve the growth of pre-and post-weaning piglets and intestinal development in post-weaning piglets[10-12].

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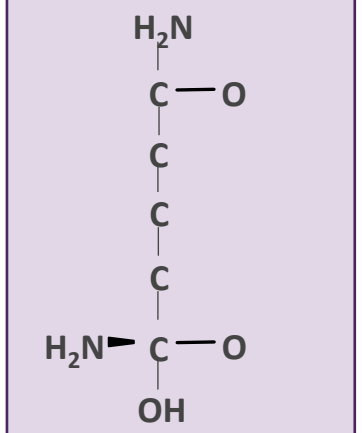
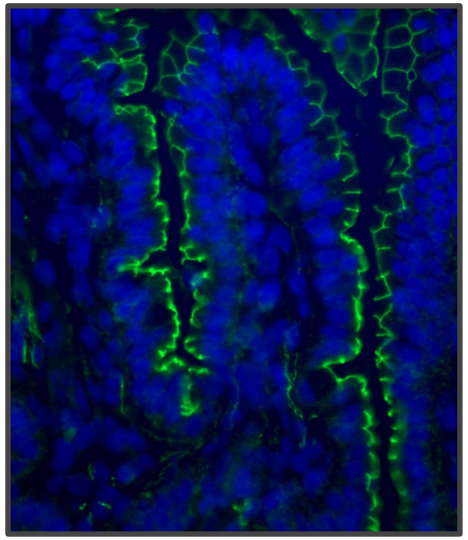
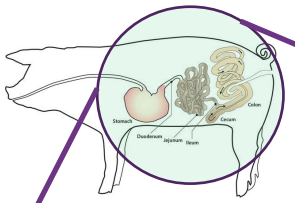
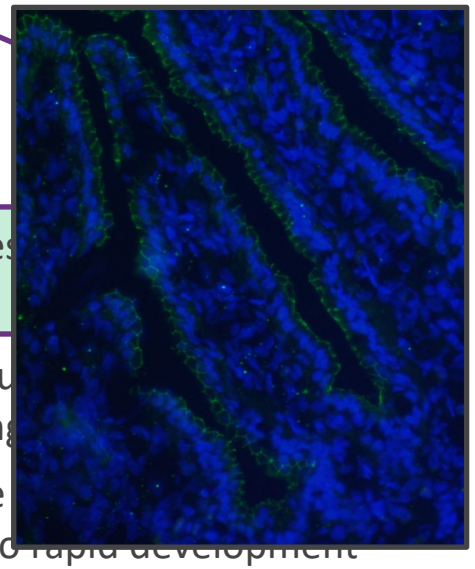
Glutamine – more than just an amino acid





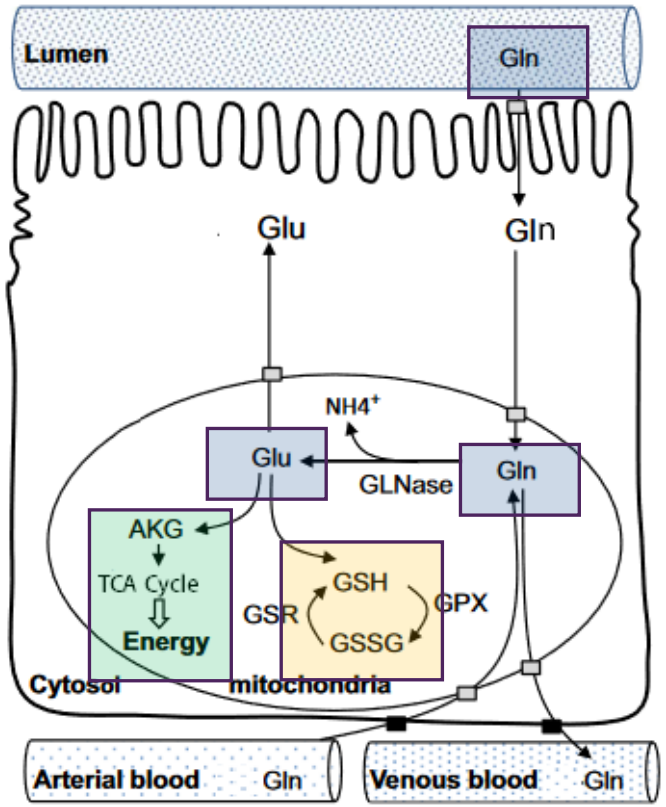




In pigs the gastrointes
rapidly after birth:

- The weight of the jeju
almost doubles during
- The cells that line the
(enterocytes) undergo rapid development

- Image 1



- Nutrient uptake
- Secretion of enzymes and immune molecules
- Passive barrier function

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Glutamine (Gln) is the primary fuel for enterocytes of the small intestine[13]

Glutamine is taken up by the enterocyte, enters the mitochondria and is converted to Glutamate (Glu)

Glutamate is converted to Alpha-KetoGlutarate (AKG), enters the TCA cycle and used to generate ATP (energy)

Glutamate can also be converted to Glutathione (GSH), an antioxidant

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Previous glutamine supplementation research in pigs

Most studies investigating glutamines effect on growth and development in pigs, have focused on the **weaning period**, results have reported that glutamine:

- Increased bodyweight
- Increased daily bodyweight gain
- Protected against intestinal atrophy by improving villus height and crypt depth
- Reduced intestinal permeability
- Prevented some negative metabolic changes associated with weaning[16]

Glutamine supplementation studies in **pre-weaning pigs** have shown that:

- Increased bodyweight, when supplemented from 7 to 21 days of age[18]
- Protected against intestinal atrophy by improving villus height[18]
- Increased daily bodyweight gain when supplemented from birth to 21 days age[10]

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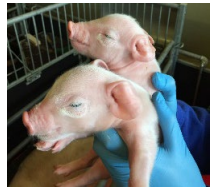

The Q (Glutamine)-PIG1 Project - (2016 – 2020)

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Hypothesis:

Oral glutamine supplementation during the early suckling period improves the bodyweight of low birthweight piglets, and is associated with changes in small intestine morphology/development and metabolism.

Aims:

To determine if oral glutamine supplementation of low birthweight piglets, during the early suckling period is associated with:

1. Improved bodyweight,
2. Morphological / developmental changes of the small intestine,
3. Changes in glutathione synthesis.

To determine if the changes observed from 1-3 are still present after supplementation has stopped.

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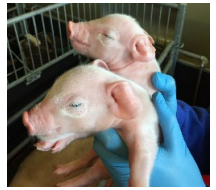

The Q-PIG1 Project – Trial design

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Birthweight Groups

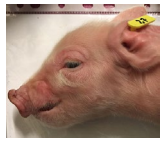
Age Groups

Supplementation Groups
(12 piglets / litter)

L-Gln	N-Gln
L-Ala	N-Ala

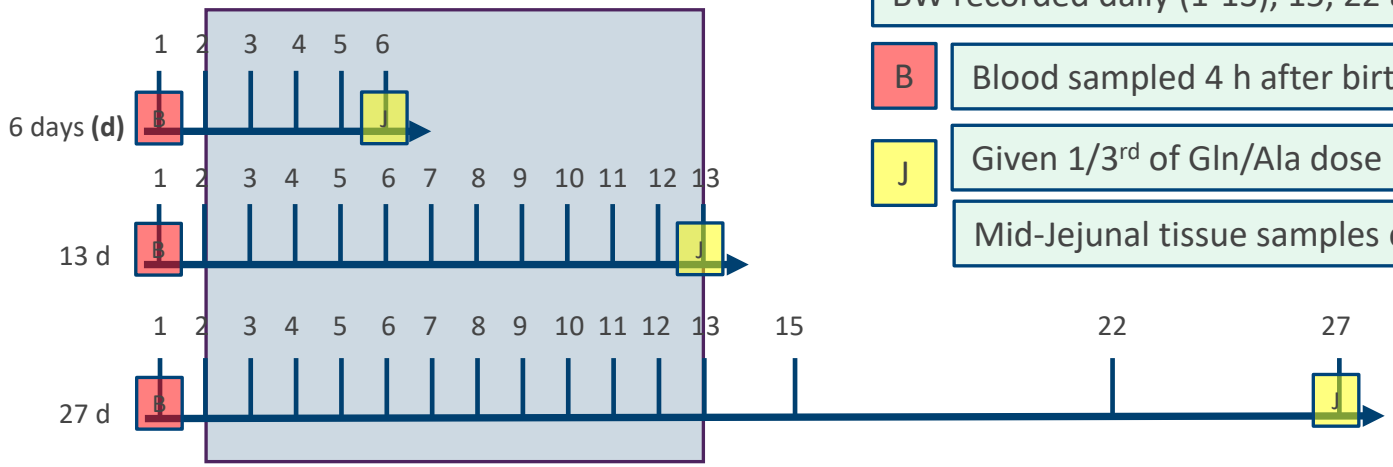
N = 12 piglets per group
Total N = 144 pigs

Low birthweight **(L)***
(0.8 – 1.2 kg)



Normal birthweight **(N)***
(1.4 – 1.8 kg)

Males: Born to gilts



BW recorded daily (1-13), 15, 22 and 27 d

B — Blood sampled 4 h after birth (1 d)

J — Given 1/3rd of Gln/Ala dose 1.5 h before euthanasia

Mid-Jejunal tissue samples collected

Supplementation period (2 – 13 d), 3 times a day at 7:00, 12:00, 17:00 h

Glutamine **(Gln)**, 1 g / kg bodyweight (**BW**) / d [18]
Alanine **(Ala)**, 1.22 g / kg BW / d (equal amount of nitrogen to Gln supplemented pigs)

***(L)**, below the lowest birthweight quartile, **(N)**; represents the middle 50th percentile, of the experimental pig facility

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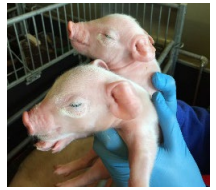

The Q-PIG1 Project - Results

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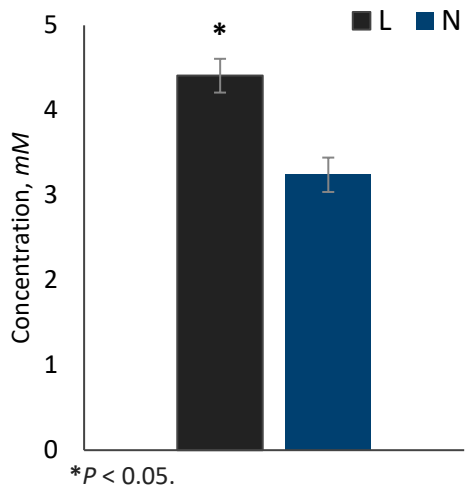
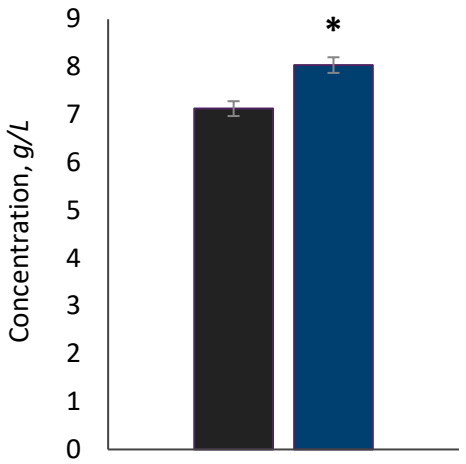
Plasma metabolites at 4 hours after birth

Inositol



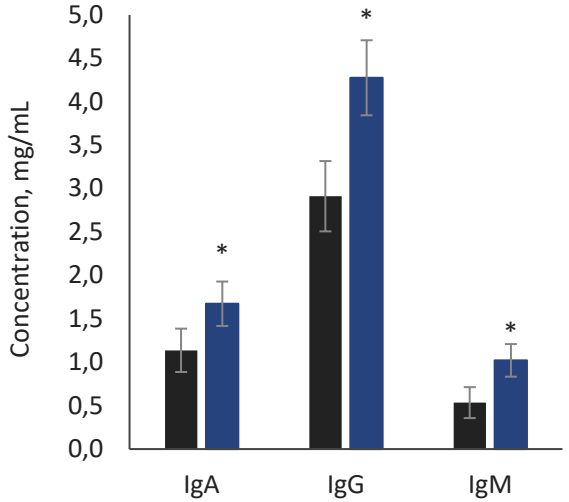
- Biomarker for low birthweight[8]

Albumin



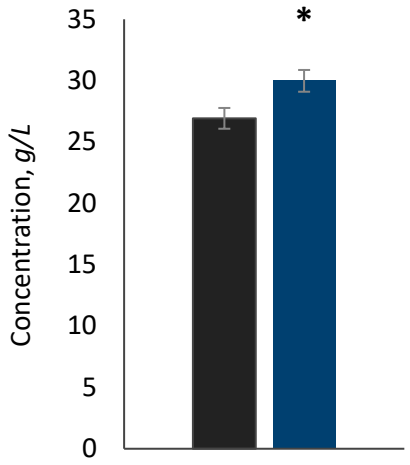
- Indicator of low birthweight[17]

Immunoglobulins (Ig)



- Ig can only be obtained from colostrum

Total protein



Taken together, these results provide strong evidence that the low birthweight piglets selected for this study are low birthweight

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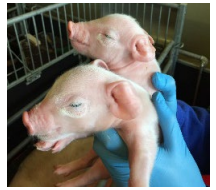

The Q-PIG1 Project - Results

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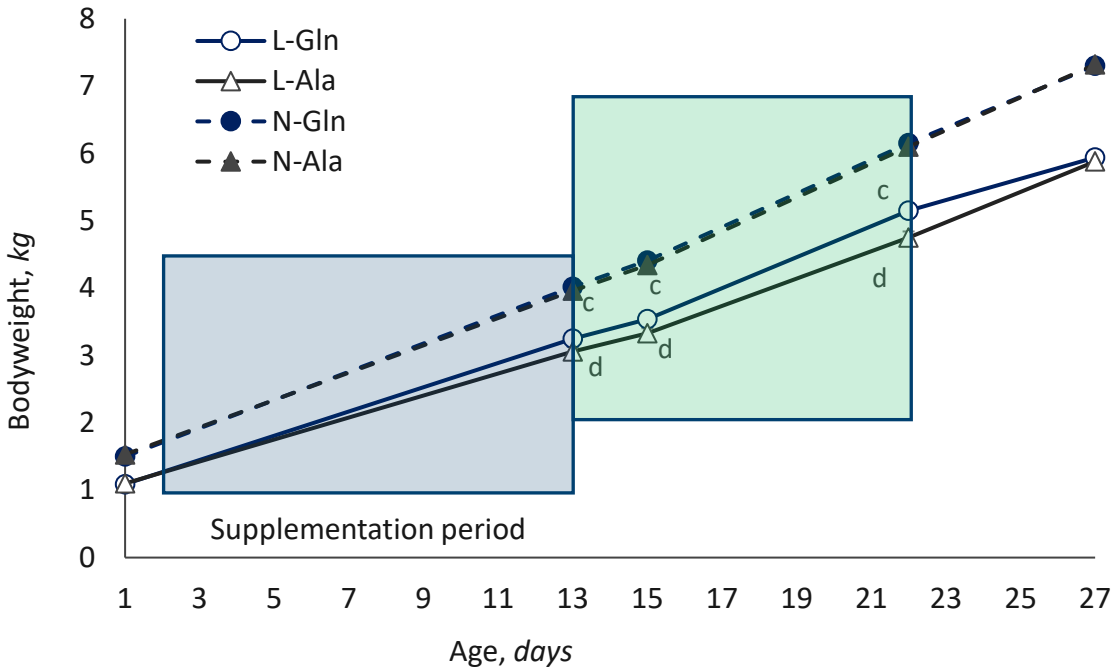
Piglet growth[11,12]

Bodyweight



Average Daily Gain

Period (g/d)	L-Gln	L-Ala	N-Gln	N-Ala	SEM
1 - 6 d	129[b]	122[b]	156[a]	156[a]	7.90
7 - 13 d	179[b]	165[b]	204[a]	200[a]	8.36
14 - 27 d	187[b]	186[b]	224[a]	223[a]	10.9

- Bodyweight and Average Daily Gain were always higher in N then L piglets
- At ages 13, 15 and 22 d L-Gln piglets were heavier than L-Ala

✓ Oral supplementation of Gln is associated with increased bodyweight in L piglets and this effect persists beyond the supplementation period

[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Ala piglets within Birthweight group ($P < 0.05$).

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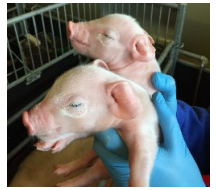

The Q-PIG1 Project - Results

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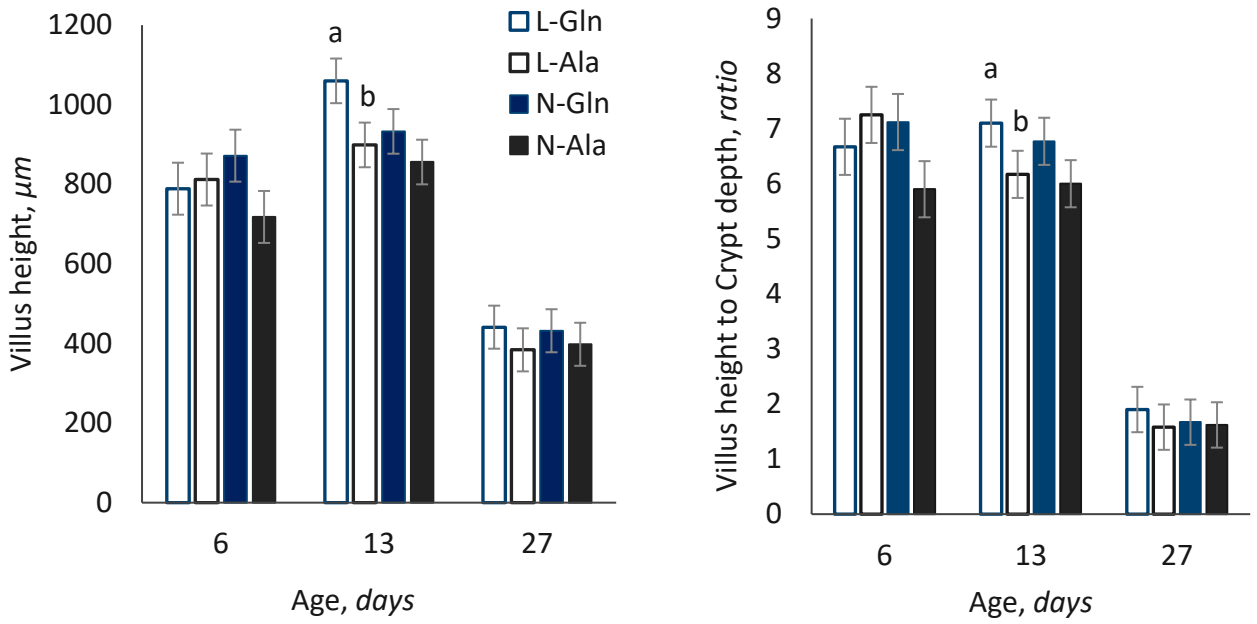
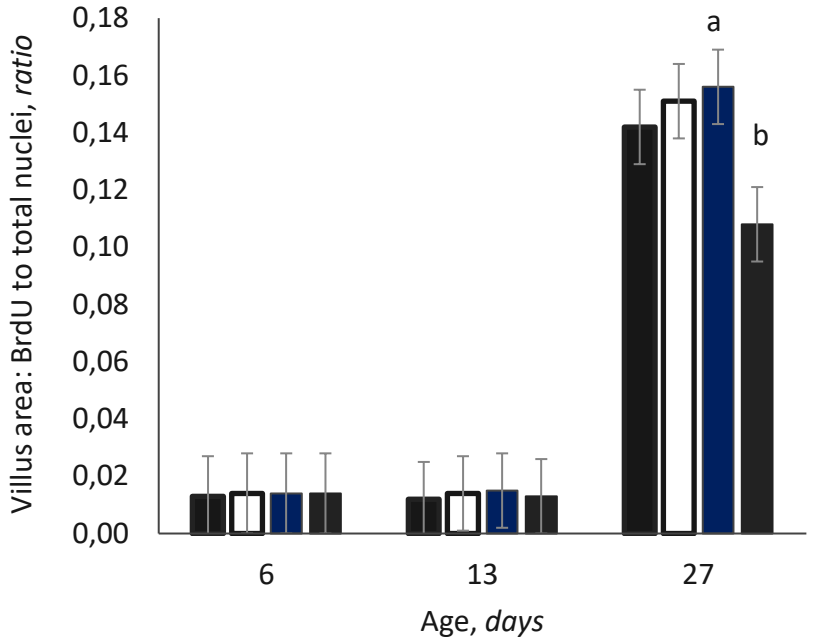
Jejunal parameters[12]

Jejunal glutathione concentrations were measured.
No differences were observed.

Villus height and Villus height to crypt depth ratio



Cell proliferation



[a,b]Different from Ala piglets within Birthweight group ($P < 0.05$).

These results suggest increased jejunal digestive and absorptive capabilities in low birthweight male piglets supplemented with glutamine compared to low birthweight alanine piglets

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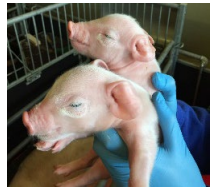

The Q-PIG1 Project - Conclusions

Oral glutamine supplementation (1g/kg BW/day) from 2 – 13 days of life to male low birthweight piglets:

- ✓ Improved bodyweight from 13 – 22 days of life compared to low birthweight controls
- ✓ Improved jejunal digestive and absorptive capabilities at 13 days of life

Open questions:

- How does glutamine improve low birthweight piglet bodyweight?
- What would happen if piglets were supplemented longer?
- What if controls were supplemented with water?
- What if a more proximal section of the small intestine was analyzed?

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MonoGutHealth Project – (2021 – 2023)

Hypothesis:

Oral glutamine supplementation during the early suckling period improves the **bodyweight** of low birthweight piglets, and is associated with changes in **glutamine and/or glucose metabolism**.

Aims:

To determine if oral glutamine supplementation of low birthweight piglets, during the early suckling period is associated with:

1. Improved bodyweight,
2. Changes in small intestine glutamine or glucose metabolism,
3. Changes in small intestine development.

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.

Catheter study: Study design

Birthweight Groups

Low birthweight **(L)*** (0.8 – 1.2 kg)

Normal birthweight **(N)*** **(1.5 – 1.9 kg)**

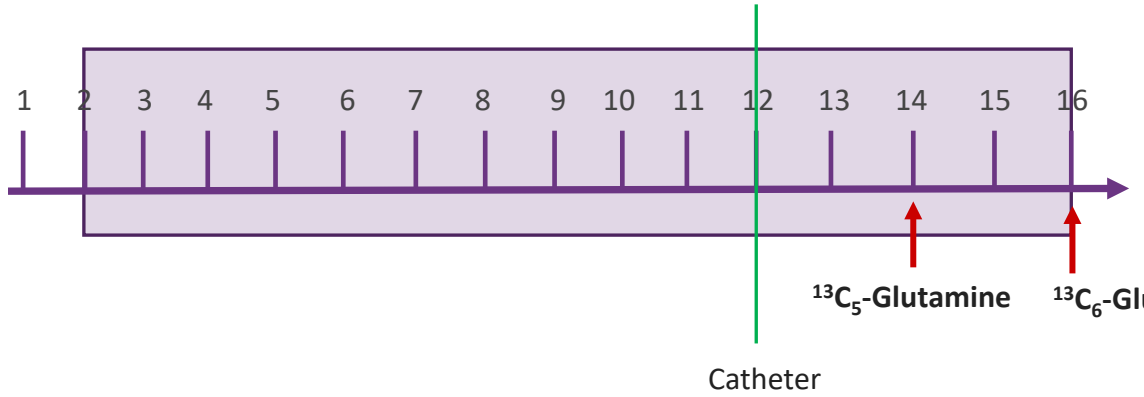
Males **Born to parity 2-9 sows**

Supplementation Groups **(14 piglets per litter)**

L-Gln	**N-Gln**
L-W	**N-W**

N = 12 piglets per group
Total N = 48 pigs

Glutamine **(Gln)**, 1 g / kg bodyweight (**BW**) / d
Water (W) (equal volume to Gln supplemented pigs)



BW recorded daily

1h separation from sow

B Every 30 min for 5 hours

Stay with litter during sampling (can suckle)

Supplementation period (2 – 16 d), 3 times a day at 7:00, 12:00, 17:00 h

***(L),** below the lowest birthweight quartile, **(N);** represents the middle 50th percentile, of the experimental pig facility

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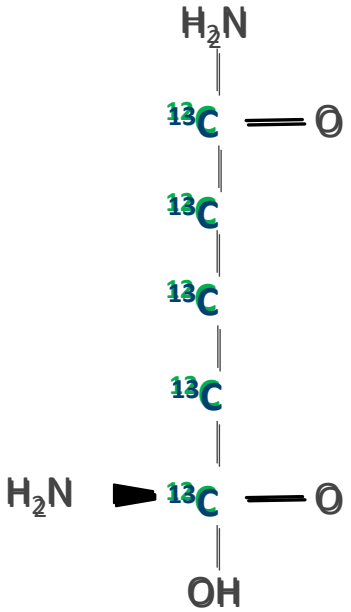
Stable isotope tracers: Where are the carbons?



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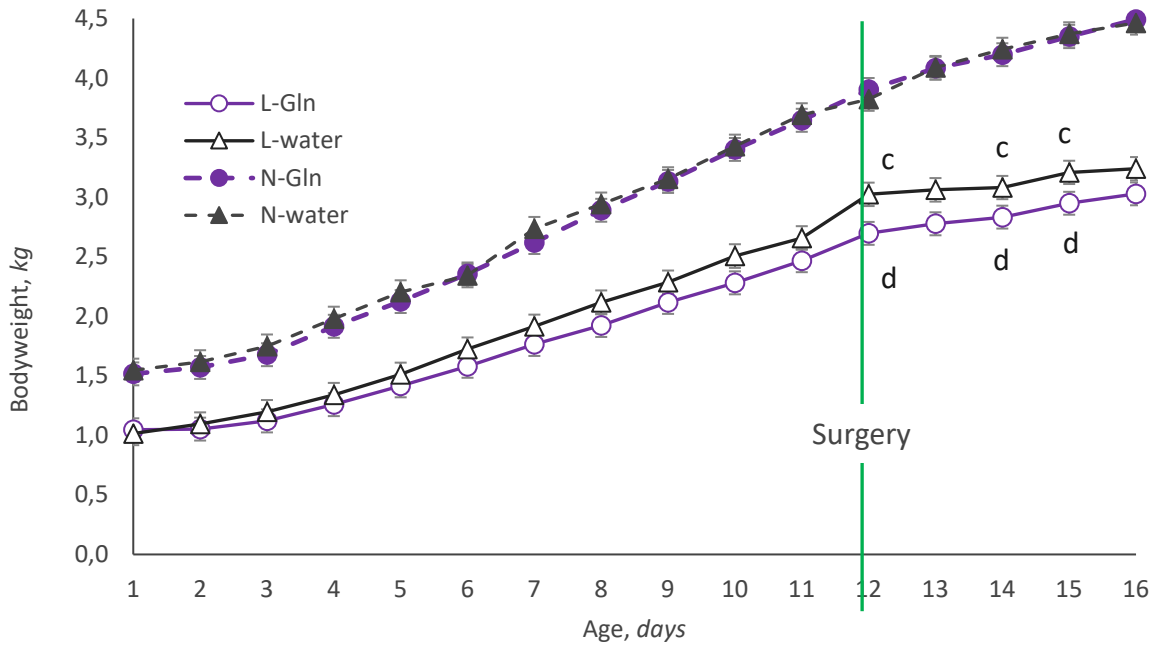
Catheter study: Piglet growth

Bodyweight



[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

Average Daily Gain

(g/d)	Period	L-Gln	L-water	N-Gln	N-water
Total	1 - 16 d	133 ± 9[b]	144 ± 9[b]	211 ± 9[a]	190 ± 9[a]
Pre-Surgery	1 - 12 d	158 ± 13[b]	180 ± 13.4[b]	222 ± 12.6[a]	206 ± 13.4[a]
Post-Surgery	13 - 16 d	25.2 ± 9[b]	19.8 ± 9[b]	39.3 ± 9[a]	42.4 ± 9[a]

- Bodyweight and Average Daily Gain were always higher in N then L piglets
- On the day of surgery (12), 14 and 15 days L-water piglets were heavier than L-Gln
- Surgery negatively impacted piglet bodyweight and average daily gain

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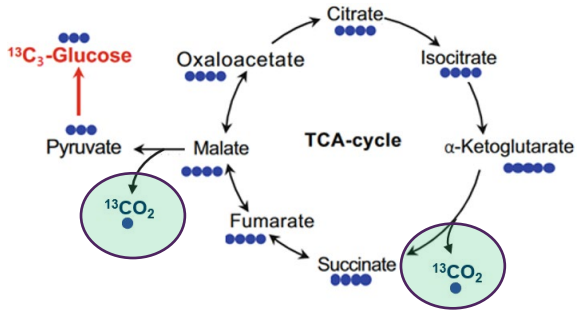
Catheter study: $^{13}C_5$-Glutamine metabolism



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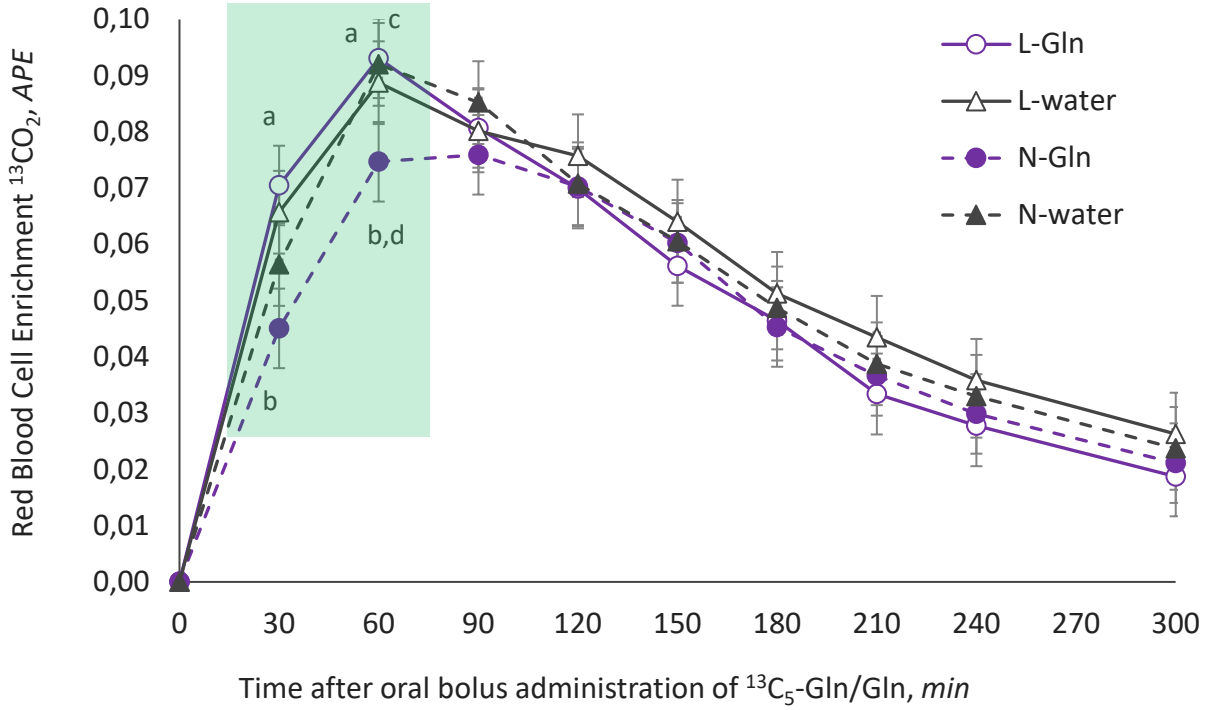
^{13}C-Carbon dioxide enrichment in Red Blood Cells



[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

Kinetic parameters

	L-Gln	L-water	N-Gln	N-water
AUC, *APE×min*	15.5 ± 1.66	16.7 ± 1.73	14.3 ± 1.66	15.8 ± 1.73
E_{max}, *min*	0.100 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
T_{max}, *min*	62.9 ± 5.54[b]	75.1 ± 5.83	80.3 ± 5.54[a]	65.0 ± 5.83

- N-Gln had a higher T_{max} than L-Gln

- N-Gln RBC $^{13}CO_2$ enrichment is lower than:
  - L-Gln at 30, 60 min
  - N-water at 60 min

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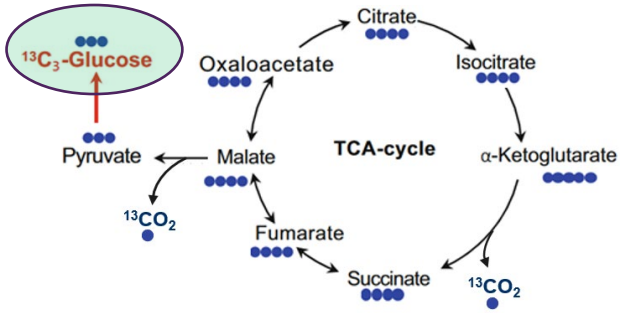
Catheter study: $^{13}C_5$-Glutamine metabolism



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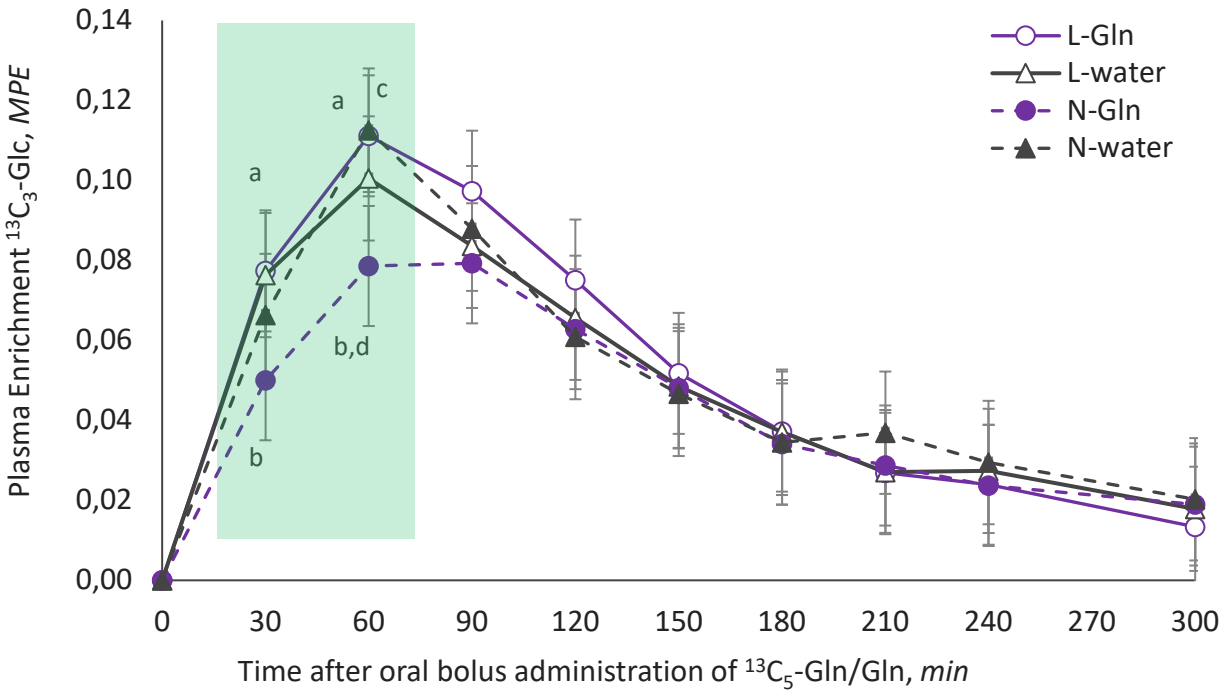
$^{13}C_3$-Glucose enrichment in Plasma



Kinetic parameters

	L-Gln	L-water	N-Gln	N-water
AUC, *MPE×min*	15.4 ± 3.47	14.9 ± 3.53	12.9 ± 3.42	13.4 ± 3.60
E_{max}, *min*	0.118 ± 0.03	0.108 ± 0.03	0.086 ± 0.03	0.125 ± 0.03
T_{max}, *min*	59.6 ± 5.00[b]	63.8 ± 5.34	73.6 ± 4.80[a]	63.3 ± 5.09

- N-Gln had a higher T_{max} than L-Gln

- N-Gln Plasma $^{13}C_3$-Glucose enrichment is lower than:
  - L-Gln at 30, 60 min
  - N-water at 60 min

[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

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Catheter study: $^{13}C_5$-Glutamine metabolism

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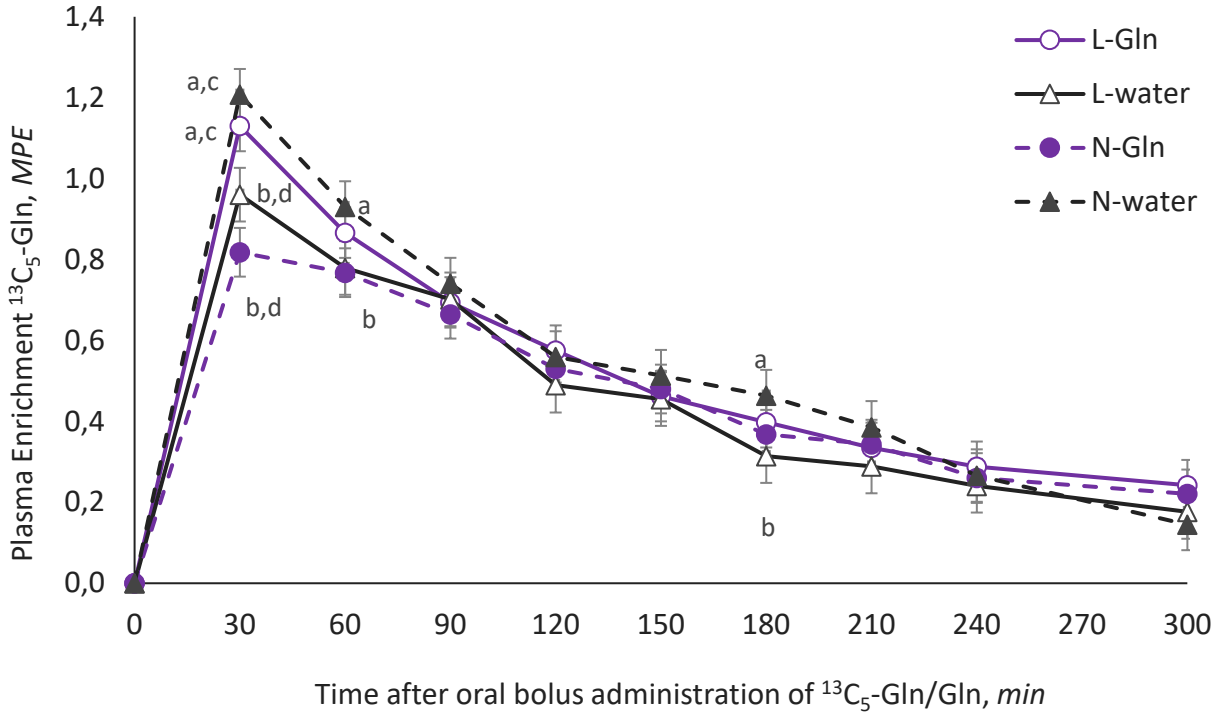
$^{13}C_5$-Glutamine enrichment in Plasma



[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

Kinetic parameters

	L-Gln	L-water	N-Gln	N-water
AUC, *MPE×min*	161 ± 14.3	141 ± 15.3	142 ± 13.8	171 ± 14.7
E_{max}, *min*	1.16 ± 0.09[a]	0.983 ± 0.100[b]	0.893 ± 0.086[b,d]	1.24 ± 0.091[a,c]
T_{max}, *min*	32.4 ± 3.71	36.5 ± 3.97	37.8 ± 3.57	28.3 ± 3.79

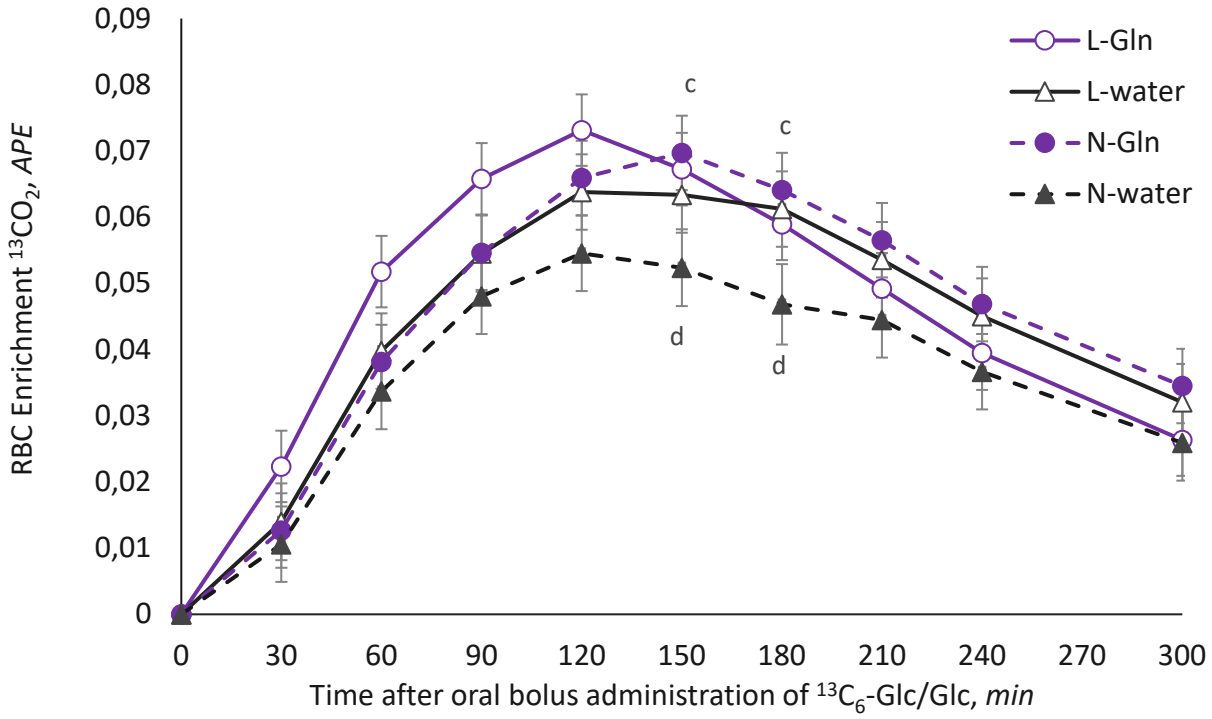
- N-Gln had a lower E_{max} than L-Gln and N-Water
- L-water had a lower E_{max} than N-Water

- L-Gln Plasma $^{13}C_5$ Glutamine enrichment is higher than:
  - N-Gln and L-water at 30 min
- N-water Plasma $^{13}C_5$ Glutamine enrichment is higher than:
  - N-Gln at 30, 60 min
  - L-water at 30, 60, 180 min

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.

Catheter study: $^{13}C_6$-Glucose metabolism

^{13}C-Carbon dioxide enrichment in Red Blood Cells



[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

Kinetic parameters

	L-Gln	L-water	N-Gln	N-water
AUC, *APE×min*	14.2 ± 7.80	13.6 ± 7.80	14.1 ± 7.80	11.2 ± 7.80
E_{max}, *min*	0.07 ± 0.008	0.07 ± 0.008	0.07 ± 0.008	0.05 ± 0.008
T_{max}, *min*	116 ± 8.02[b]	125 ± 8.47	143 ± 8.32[a]	122 ± 8.47

- N-Gln had a higher T_{max} than L-Gln

- N-Gln RBC $^{13}CO_2$ enrichment is higher than:
  - N-water at 150 and 180 min

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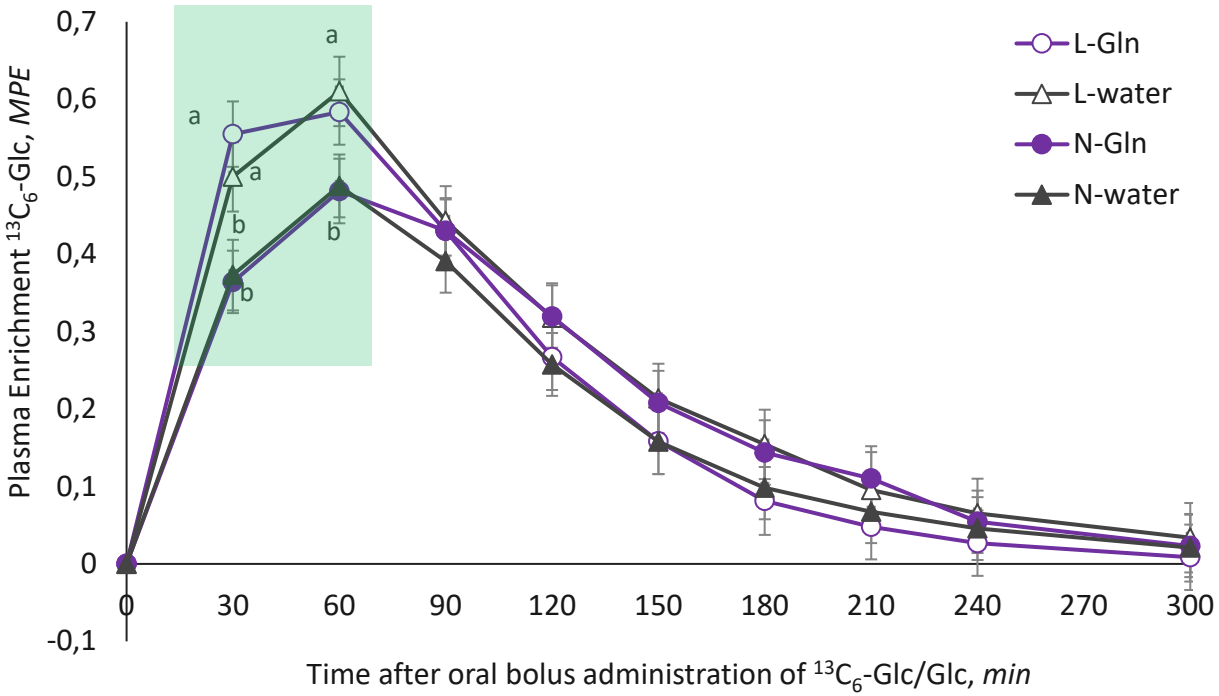
Catheter study: $^{13}C_6$-Glucose metabolism

$^{13}C_6$-Glucose enrichment in Plasma



[a,b]Different from N piglets within Supplementation group ($P < 0.05$).

Kinetic parameters

	L-Gln	L-water	N-Gln	N-water
AUC, *MPE×min*	68.0 ± 7.80	75.4 ± 8.38	68.0 ± 7.50	59.7 ± 7.64
E_{max}, *min*	0.701 ± 0.07	0.656 ± 0.08	0.537 ± 0.07	0.558 ± 0.07
T_{max}, *min*	47.5 ± 5.28	50.3 ± 5.56	58.1 ± 5.05	50.7 ± 5.08

- No differenced in kinetic parameters

- L-Gln Plasma $^{13}C_6$-Glucose enrichment is higher than:

  N-Gln at 30 min

- L-water Plasma $^{13}C_6$-Glucose enrichment is higher than:

  N-Water at 30, 60 min

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Tissue study: Study design

Birthweight Groups

Low birthweight **(L)*** (0.8 – 1.2 kg)

Normal birthweight **(N)*** **(1.5 – 1.9 kg)**

Males
Born to parity 2-9 sows

Supplementation Groups
(14 piglets per litter)

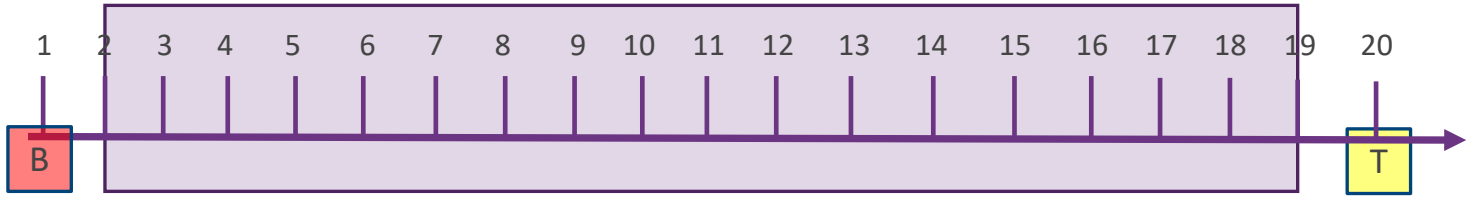
L-Gln	**N-Gln**
L-W	**N-W**

N = 12 piglets per group
N-Gln = 14 piglets
Total N = 50 pigs

Glutamine **(Gln)**, 1 g / kg bodyweight (**BW**) / d
Water (W) (equal volume to Gln supplemented pigs)

BW recorded daily

Supplementation period (2 – 16 d), 3 times a day at 7:00, 12:00, 17:00 h



Blood sampled at 4 h after birth (metabolites)

Injected with $^{2}H_{5}$-Phenylanine 1h prior to measure protein synthesis

Intestine tissue sampled

***(L),** below the lowest birthweight quartile, **(N);** represents the middle 50th percentile, of the experimental pig facility

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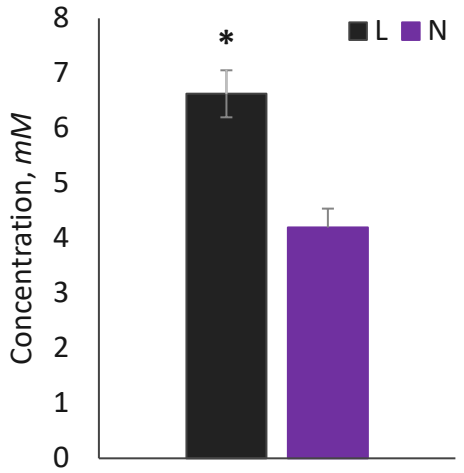
Tissue study: Plasma metabolites

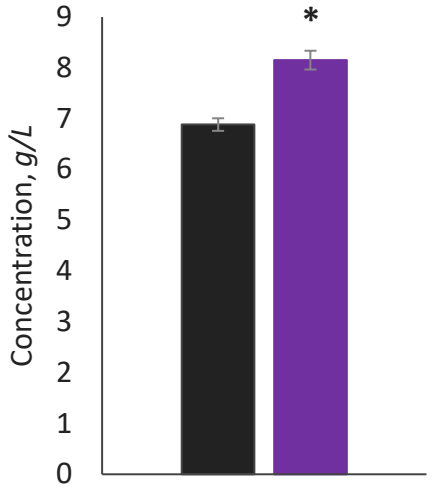
Plasma metabolites at 4 hours after birth

Inositol



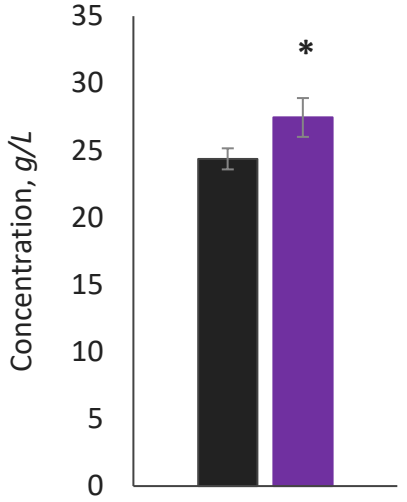
- Biomarker for low birthweight[8]

Albumin

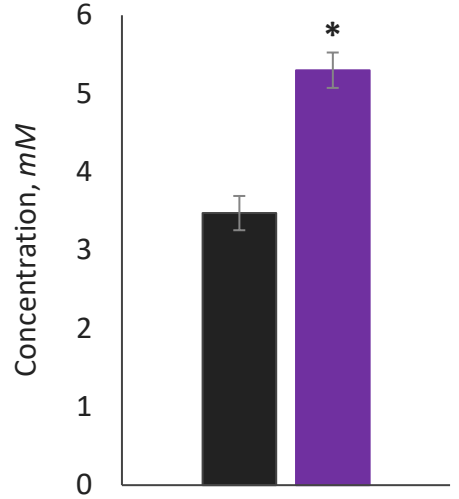


- Indicator of low birthweight[17]

Total protein



Glucose



- Indicator of colostrum intake

*$P < 0.05$, ***N*** = 36 (L = 27, N = 29).

Taken together, these results provide strong evidence that the low birthweight piglets selected for this study are low birthweight

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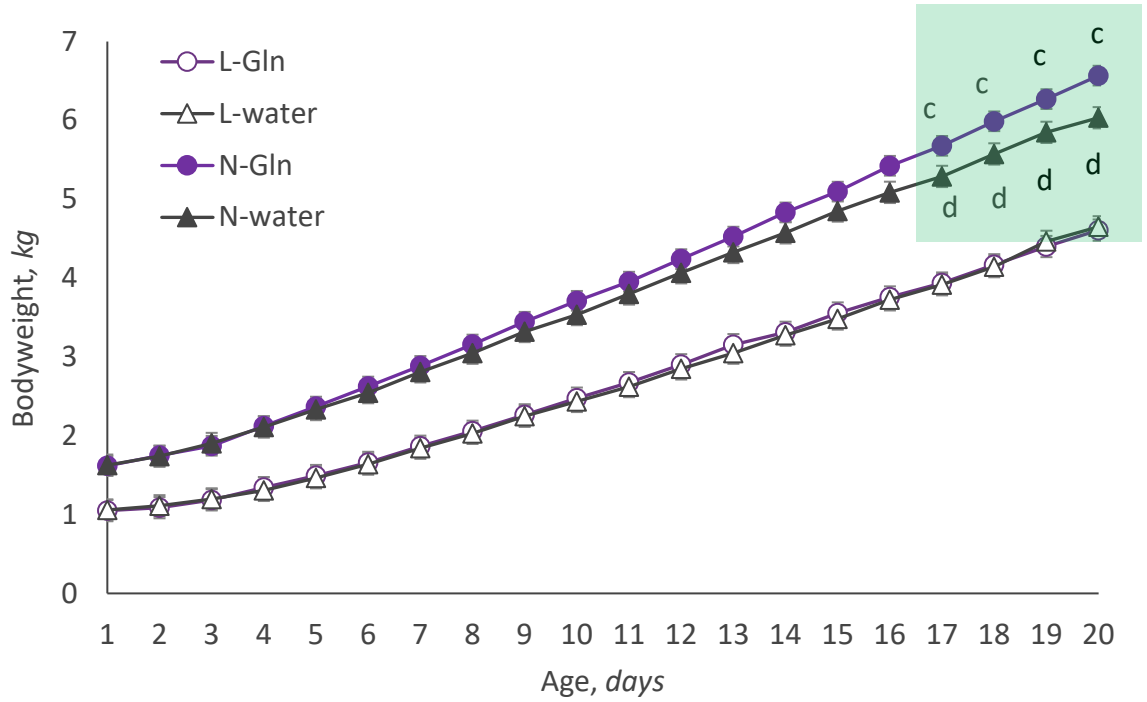
Tissue study: Piglet growth

Bodyweight



- From 17 – 20 d, N-Gln piglets were heavier than N-water

[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

Average Daily Gain

Period (g/d)	L-Gln	L-water	N-Gln	N-water	SEM
1 - 20 d	190[b]	200[b]	264[a,c]	236[a,d]	13

- Bodyweight and Average Daily Gain were always higher in N then L piglets
- Average daily gain was higher in N-Gln than N-Water

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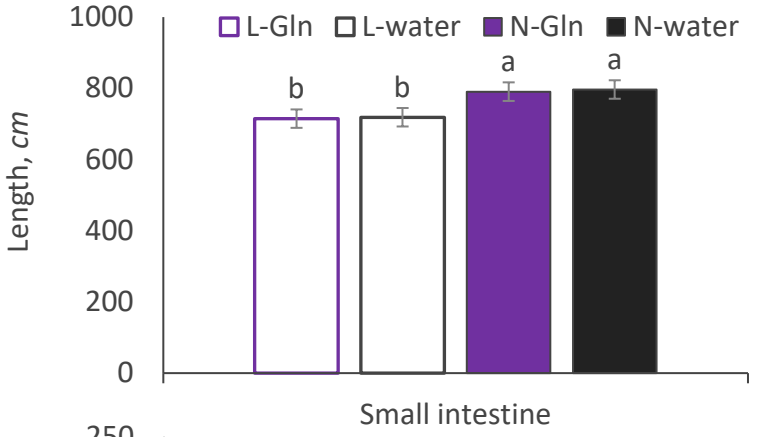
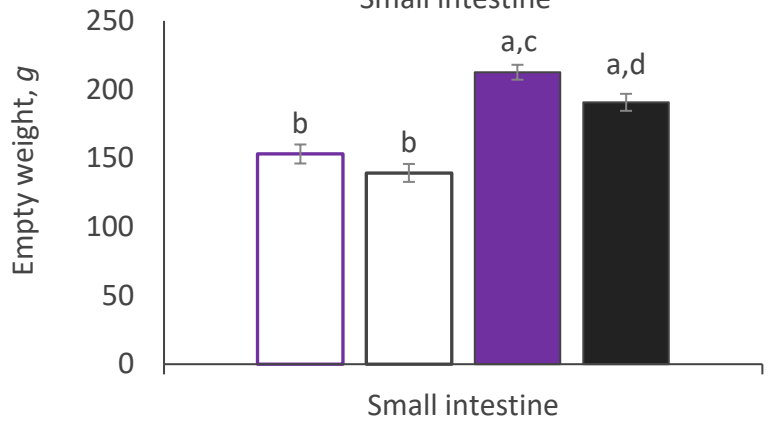
Tissue study: Intestinal parameters

Red blood Cell Glutathione concentrations were measured.
No differences were observed.

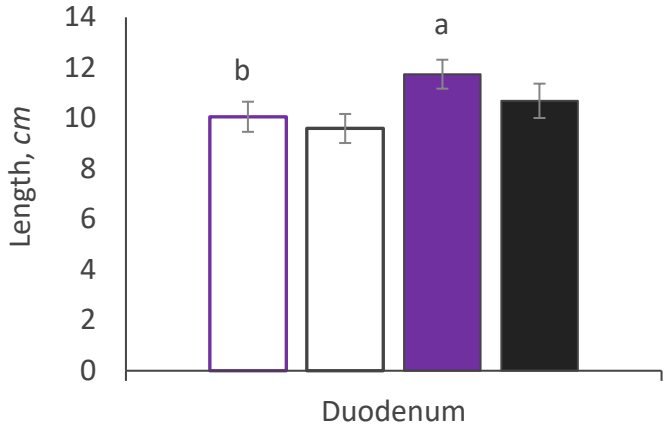
monogutthealth

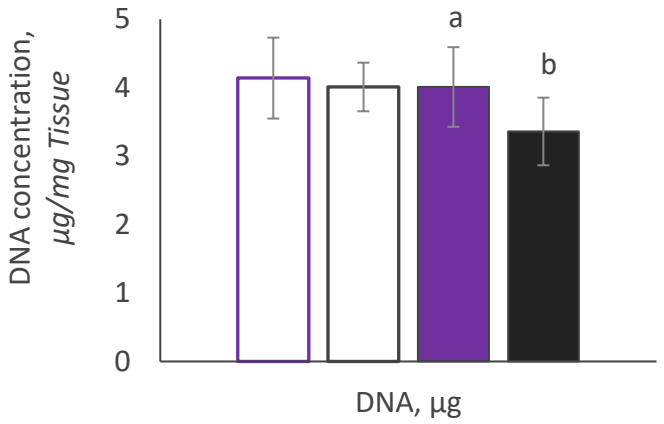
Small intestine (duodenum + jejunum + ileum)

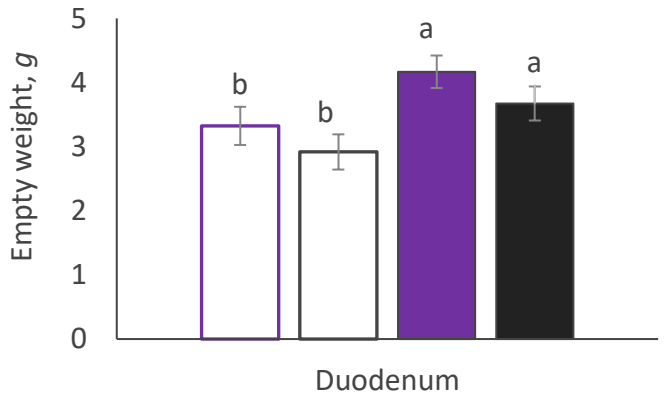


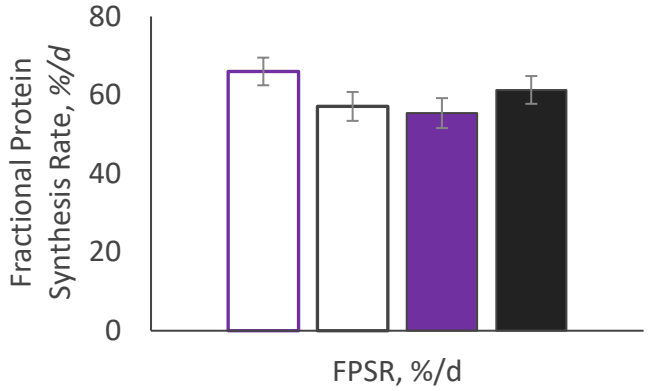


Duodenum









[a,b]Different from N piglets within Supplementation group ($P < 0.05$).
[c,d]Different from Water piglets within Birthweight group ($P < 0.05$).

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Overall Conclusions and Questions:

From the studies we have conducted:

1. Glutamine cannot be considered as a reliable supplement for improving low birthweight piglet growth
    - **Q-PIG1**: L birthweight piglets supplemented with Gln were heavier than Ala supplemented
    - **MGH Catheter**: L birthweight piglets supplemented with water were heavier than Gln supplemented
    - **MGH Tissue**: N birthweight piglets supplemented with Gln were heavier than Water supplemented
2. **MGH Catheter:** Observed changes in glutamine and glucose metabolism were not associated with birthweight. Slower enrichment in N-Gln than L-Gln….. ***Why?***
3. **MGH Tissue**: Increased bodyweight and average daily gain is associated with a heavier small intestine and higher duodenal DNA concentration in N-Gln than N-water….. ***It worked?***

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Q-PIG1

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8th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2025)

15 – 18 September 2025

Rostock-Warnemünde, Germany

Research Institute for Farm Animal Biology

Freie Universität Berlin

Universität Rostock Traditio et Innovatio

Christian-Albrechts-Universität zu Kiel

THANK YOU

Do you have any questions?

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Image 1: Beaumont, M., Blachier, F. (2020). Amino Acids in Intestinal Physiology and Health. In: Wu, G. (eds) Amino Acids in Nutrition and Health. Advances in Experimental Medicine and Biology, vol 1265. Springer, Cham. https://doi.org/10.1007/978-3-030-45328-2_1

Image 2: Nguyen, Trang TT, et al. "Methodological Approaches for Assessing Metabolomic Changes in Glioblastomas." Autophagy and Cancer: Methods and Protocols (2022): 305-328.

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