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Session 30 ***Neonatal survival in pigs***

Effect of oral glutamine supplementation in the early neonatal phase on growth, milk intake and plasma metabolites of low birth weight piglets

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Consequences of low birth weight

Low survival rate

Slow growth

Altered metabolism and development

Birth weight (kg)	Pre-weaning survival rate (%) [1,2]
< 0.6	15
< 1.0	42
1.0 – 1.2	73
> 1.2	82
> 1.6	95

[1] Quiniou et al 2002; [2] Declerck et al 2016.

Glutamine (Gln)

- A functional and conditionally essential amino acid (AA) [1]
- Abundant in sow's milk [2,3]
- A major energy source for rapidly dividing cells [4]
- Involved in energy and AA metabolism [5]
- Regulates key metabolic pathways related to growth, health and immunity [5-7]
- Most of previous studies focused on weaning and post-weaning pigs [8-11]

[1] Wu et al 2013; [2] Hurley 2015; [3] Wu et al 1994; [4] Labow et al 2000; [5] Zhu et al 2015;
[6] Ban et al 2010; [7] Xi et al 2012; [8] Yi et al 2005; [9] Kitt et al 2002; [10] Yoo et al 1997;
[11] Haynes et al 2009

Hypothesis and objective

Hypothesis:

Dietary Gln supplementation in the early neonatal period might be beneficial for LBW piglets

Objective:

To determine the influence of dietary Gln supplementation during the early neonatal period on the growth performance and plasma metabolites in LBW piglets

Experimental design – animals

- 48 pairs of male litter mates (German Landrace)
- Each pair: one LBW (0.8 – 1.2 kg) and one NBW (1.4 – 1.8 kg, birth weight control) piglets
- Born to gilts
- 10-20 piglets/litter at birth
- Standardized to 12 piglets/litter within 24 h after farrowing
- Nursed by the sows

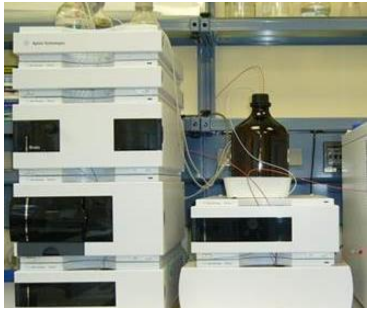
Experimental design – treatments

GLN-LBW and GLN-NBW piglets (*n* = 12/group)
ALA-LBW and ALA-NBW piglets (*n* = 12/group)
5 d

GLN-LBW and GLN-NBW piglets (*n* = 12/group)
ALA-LBW and ALA-NBW piglets (*n* = 12/group)
12 d

- ❑ 24 h after birth, the pairs of piglets were randomly assigned to Gln or Alanine (Ala) treatment
- ❑ 24 LBW and 24 NBW piglets received Gln (1 g/kg BW/day, ≈ 70% of dietary Gln intake from sow's milk, **GLN-LBW** and **GLN-NBW**)
- ❑ 24 LBW and 24 NBW piglets received Ala (1.22 g/kg BW/day, treatment control, isonitrogenous to Gln, **ALA-LBW** and **ALA-NBW**)
- ❑ 3 times a d at 7:00, 12:00, 17:00, 1-12 days of life (birth = 0 day)
- ❑ 2 age classes, **5 d** (4 groups, *n* = 12/group) and **12 d** (4 groups, *n* = 12/group)

Experimental design – measurement



- Body weight: at birth and daily
- IUGR (intrauterine growth restriction) score: at birth [1]
- Crown-rump length and abdominal circumference: at birth, 5 d, 7 d and 12 d
- Colostrum/milk composition: at 2 h, 24 h, 7 d and 12 d after farrowing
- Milk intake: 11-12 d (*i.p.* injection of D_2O, 0.2 mL/kg BW)
- Plasma metabolites, immunoglobulins and amino acids: at 4 h, 5 d and 12 d

We measured:

Liver function: ALT, AST, albumin, bilirubin

Metabolites: cholesterol, fructose, glucose, urea, lactate, NEFA, total protein, triglycerides, inositol (4 h only)

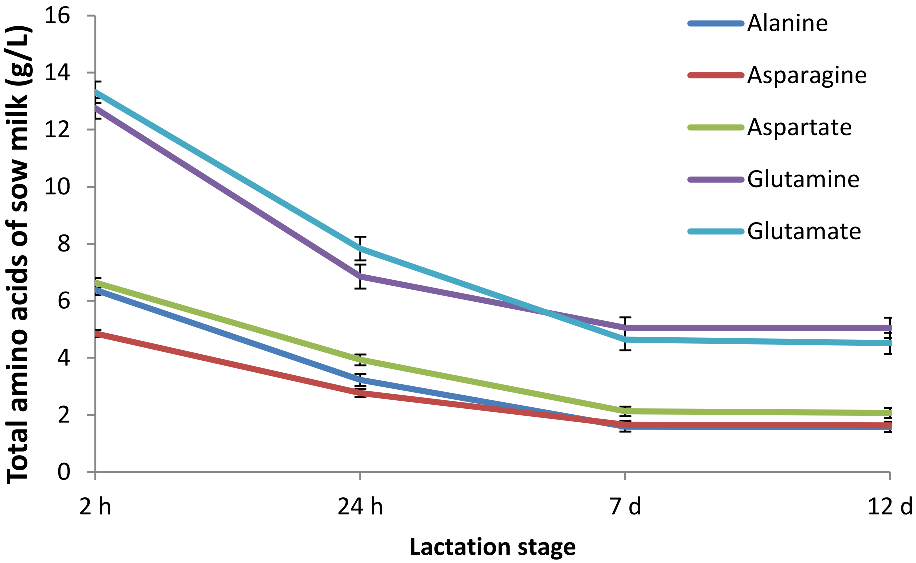
Immunoglobulins: IgA, IgG, IgM, 4 h only

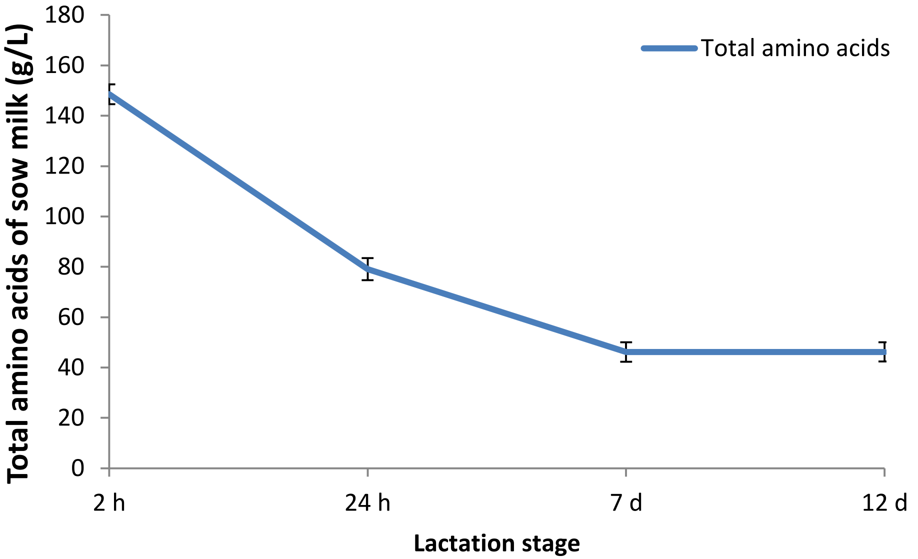
AAs: Gln, Ala, all other proteinogenic AAs, carnosine,

[1] Hales et al 2013

ALT, alanine aminotransferase; AST, aspartate aminotransferase, NEFA, non-esterified fatty acids

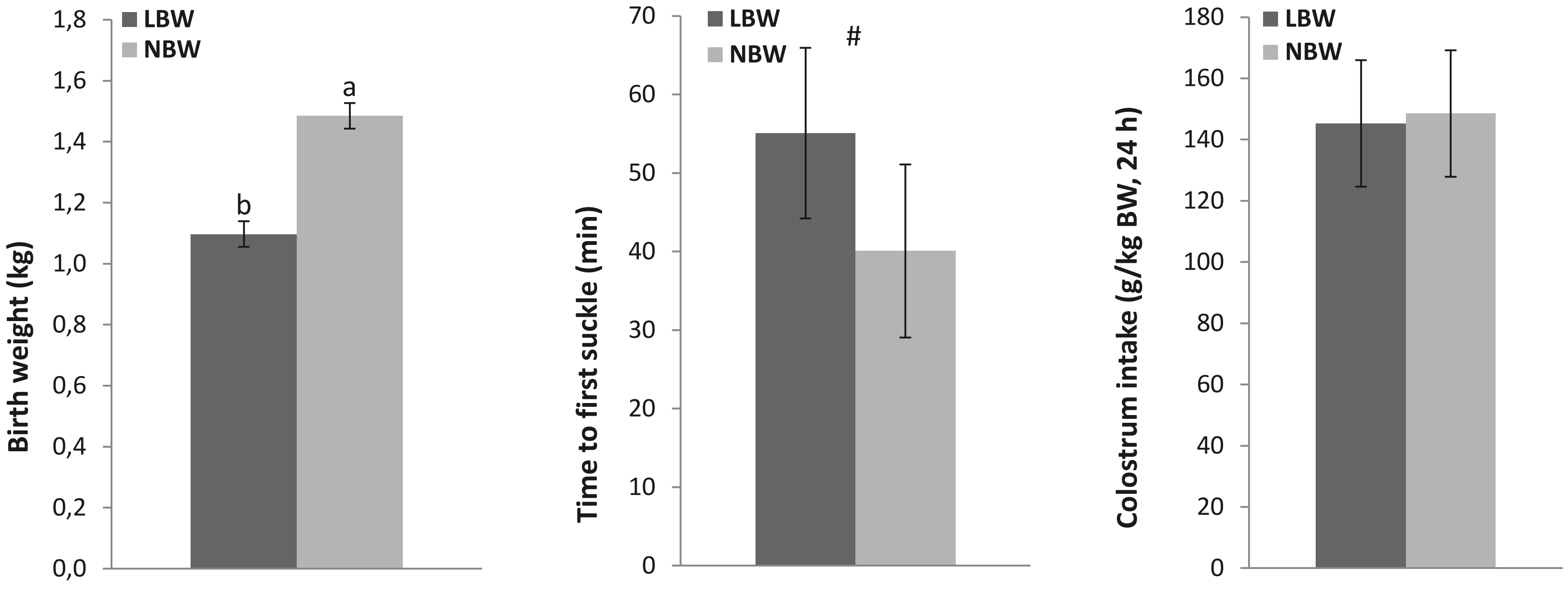
Results – milk amino acids





n = 8 / lactation stage

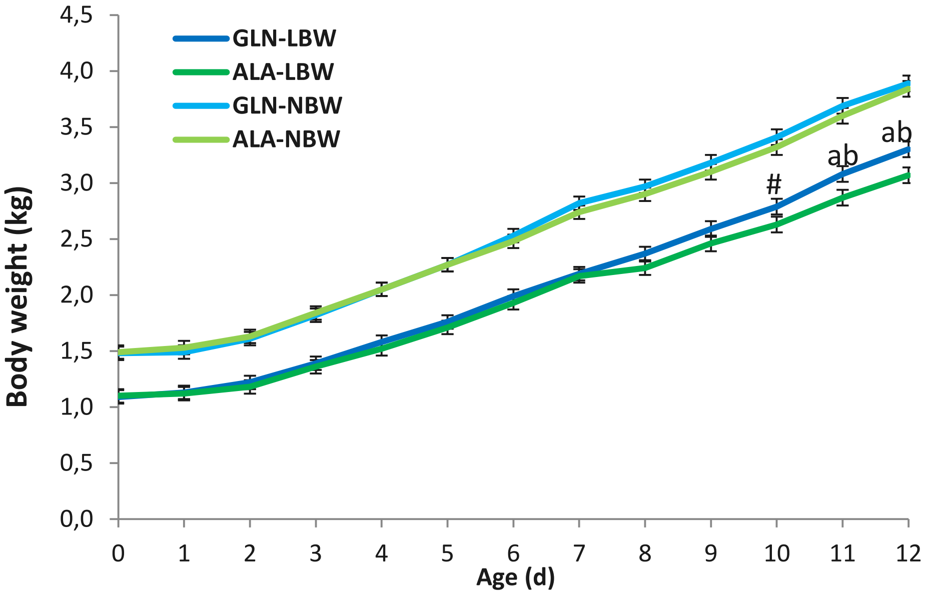
Results – birth weight, time to first suckle and colostrum intake (LBW vs. NBW)

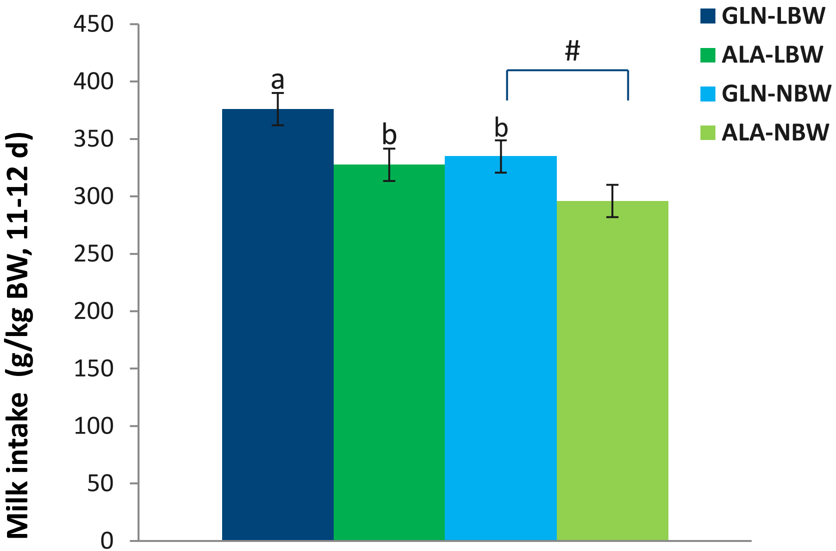


- ❑ **LBW piglets were lighter than NBW piglets at birth;**
- ❑ **LBW piglets needed more time to start suckling than NBW piglets.**

n = 48 / group
a-b Labeled columns without a common letter differ, *P* < 0.05
Labeled columns tend to differ, *P* < 0.1

Results – body weight and milk intake





- ❑ **LBW piglets were lighter than NBW piglets in both treatment groups in the first 12 d of life;**
- ❑ **GLN-LBW piglets were heavier than ALA-LBW at 10, 11 and 12 d, BW of GLN-NBW and ALA-NBW piglets did not differ;**
- ❑ **GLN piglets consumed more milk than ALA piglets in both birth weight groups at 11-12 d of life.**

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Body weight: *n* = 24 / group (0 - 5 d), *n* = 12 / group (6 - 12 d); Milk intake: *n* = 12 / group
[a-b] Labeled columns/markers without a common letter differ, *P* < 0.05
[#] Labeled columns/markers tend to differ, *P* < 0.1

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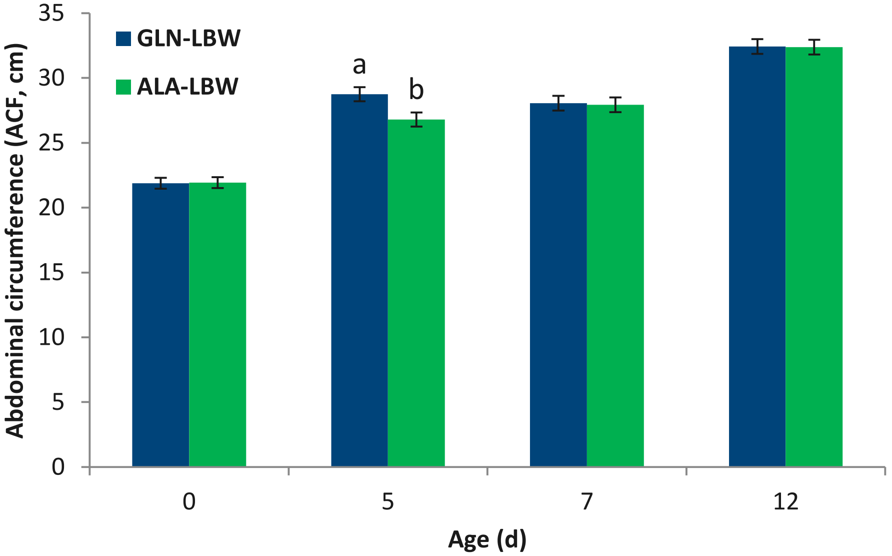
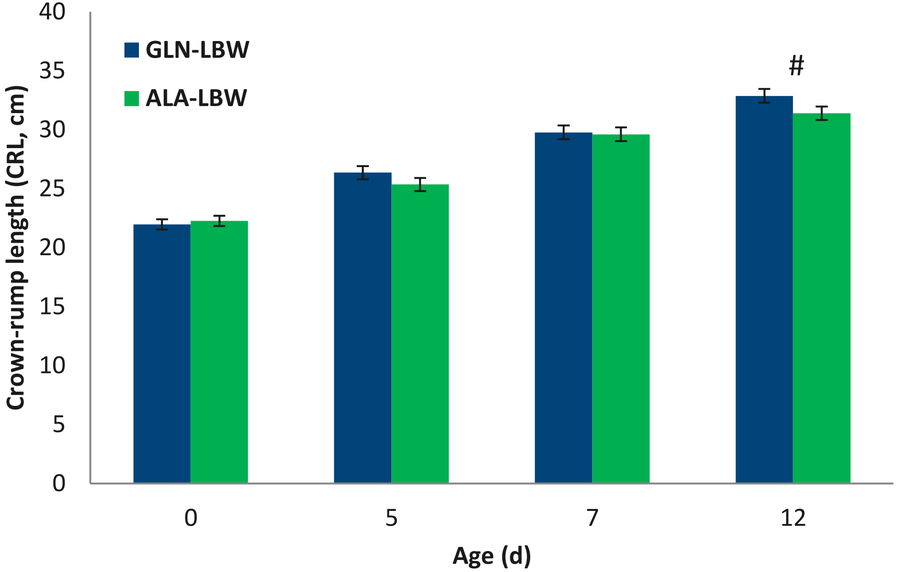
Results – body measurements (GLN-LBW vs. ALA-LBW)

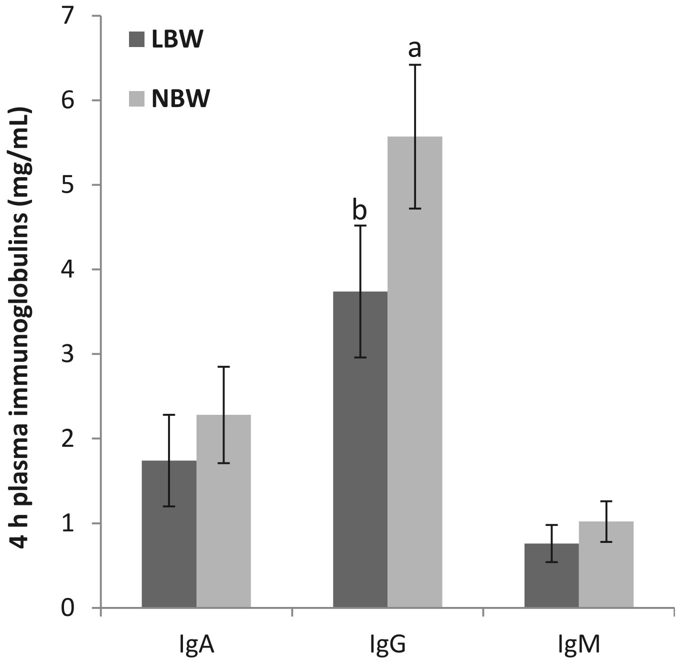


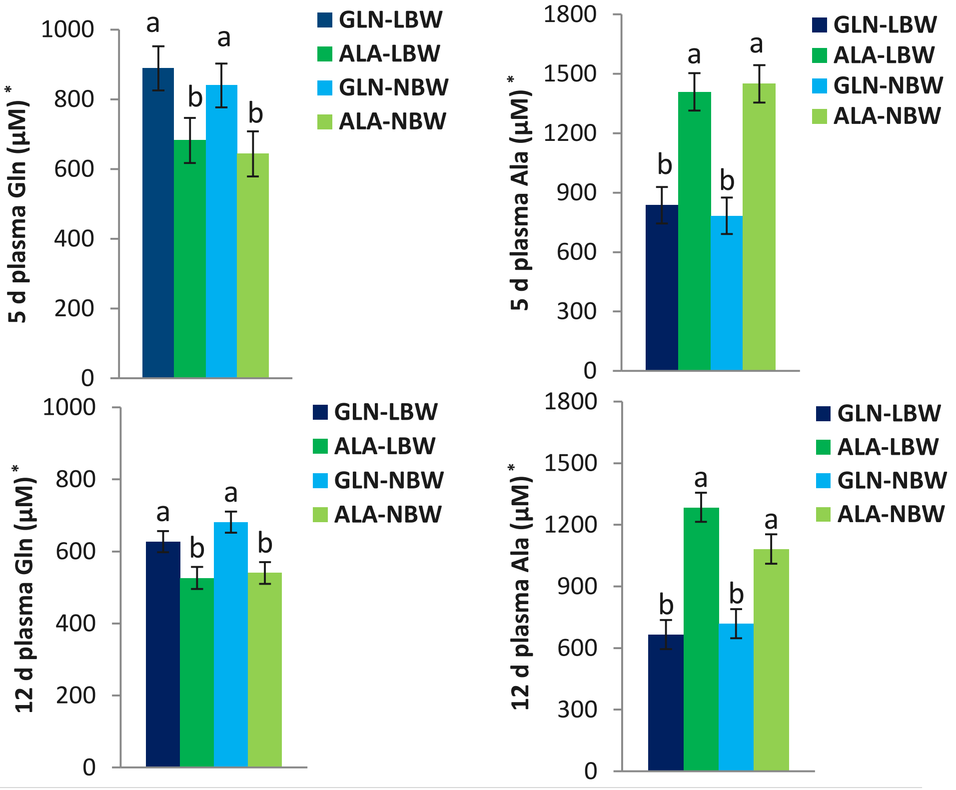


- ❑ **ACF and CRL were greater in GLN-LBW piglets at 5 and 12 d, respectively, compared with ALA-LBW piglets;**
- ❑ **ACF and CRL did not differ in GLN-NBW vs. ALA-NBW;**
- ❑ **ACF and CRL were always greater in NBW than LBW piglets in both treatment groups.**
- ❑ **BMI, ponderal index and rectal temperature did not differ in GLN-LBW vs. ALA-LBW and GLN-NBW vs. ALA-NBW;**

n = 24 / group (0 d), n = 12 / group (5, 7, 12 d)
[a-b] Labeled columns without a common letter differ, $P < 0.05$
[#] Labeled columns tend to differ, $P < 0.1$

Results – plasma immunoglobulins (4 h), Gln and Ala (5, 12 d)

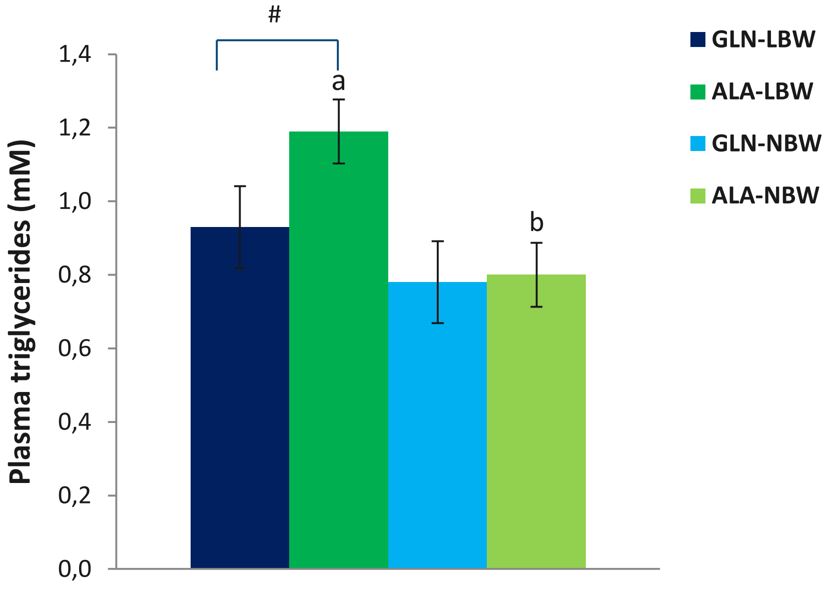


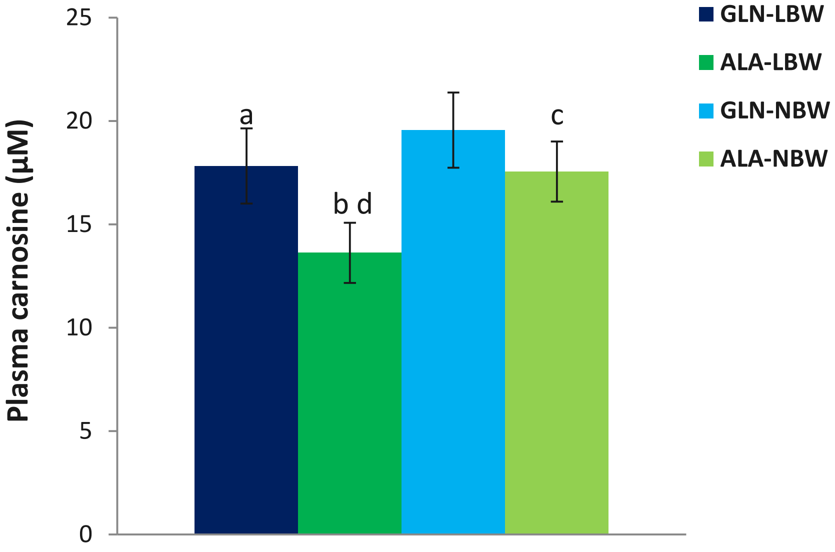


- ❑ LBW piglets had lower plasma IgG levels than NBW at 4 h after birth;
- ❑ Gln and Ala treatments increased plasma Gln and Ala concentrations.

n = 35 / LBW, *n* = 36 / NBW
n = 12 / GLN-LBW, 12 / ALA-LBW, 12 / GLN-NBW, 12 / ALA-NBW
* Plasma Gln and Ala were measured 2 h after dosing AA supplementation
a-b Labeled columns without a common letter differ, $P < 0.05$

Results – 5 d plasma metabolites





- ❑ **ALA-LBW had higher plasma triglyceride but lower plasma carnosine levels than GLN-LBW and ALA-NBW**

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n = 12 / group
[a-b] Labeled columns without a common letter differ, *P* < 0.05
[c-d] Labeled columns without a common letter differ, *P* < 0.05
[#] Labeled columns tend to differ, *P* < 0.1

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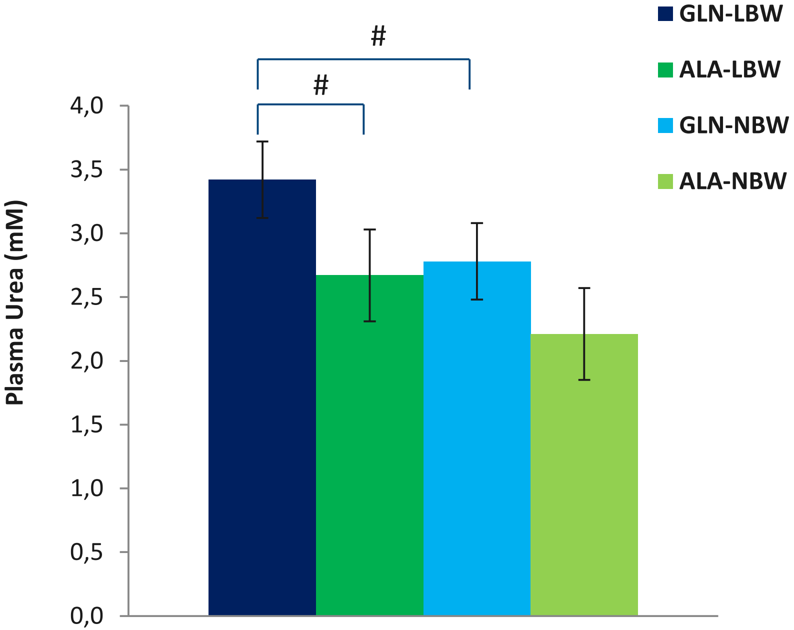
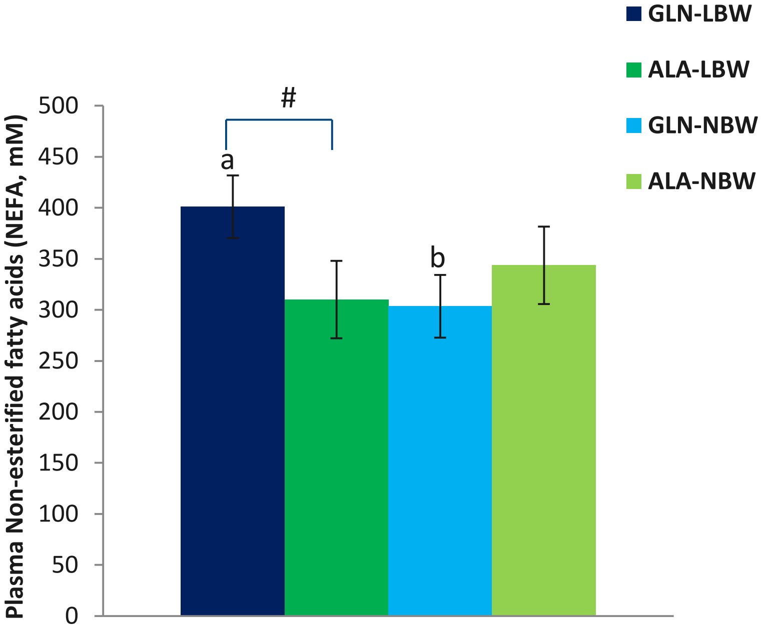
Results – 12 d plasma metabolites





- ❑ **GLN-LBW had higher plasma urea and NEFA levels than ALA-LBW and GLN-NBW**

n = 12 / group

a-b Labeled columns without a common letter differ, $P < 0.05$

Labeled columns tend to differ, $P < 0.1$

Conclusion

Gln supplementation to LBW piglets during the first 12 d of life:

- ❑ Moderately improved growth and milk intake

- ❑ Appeared to normalize lipid metabolism of LBW piglets, possibly associated with normalization of plasma carnosine levels

Thank you !

Questions are welcome !

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Birth weight distribution

Birth weight (kg)	Proportion (%)
< 0.8	5.0
0.8-1.2	35.9
1.2-1.4	30.5
1.4-1.8	26.6
>1.8	2.0

Data source: German Landrace gilts and their progeny in FBN (30 gilts and 452 piglets)

IUGR score

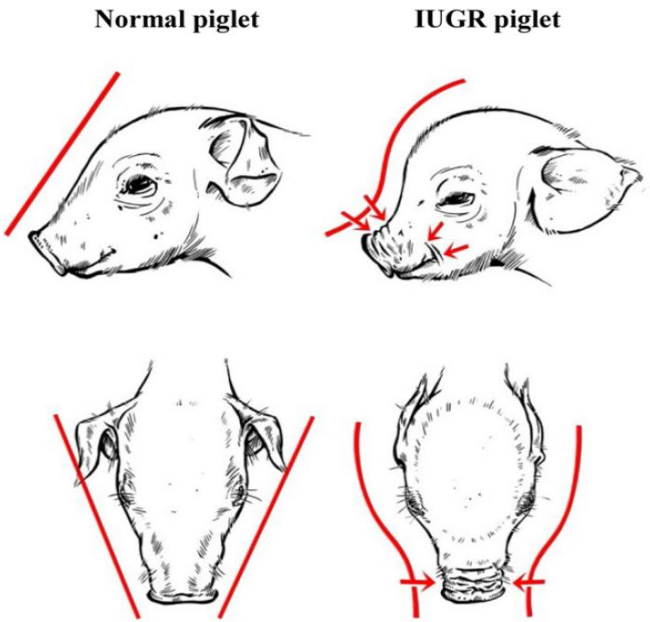
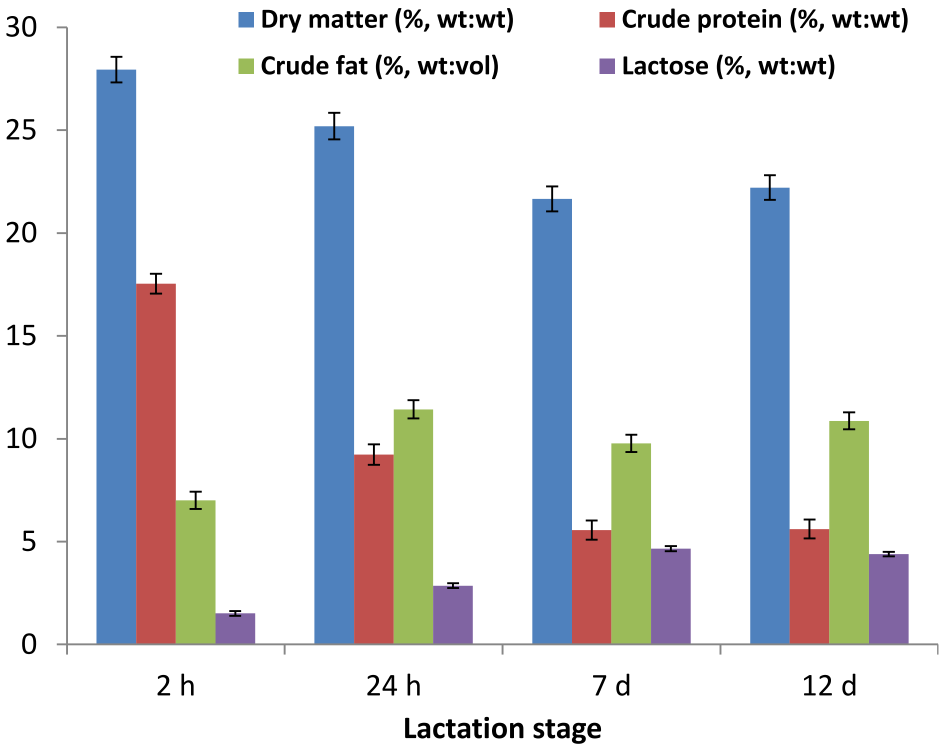


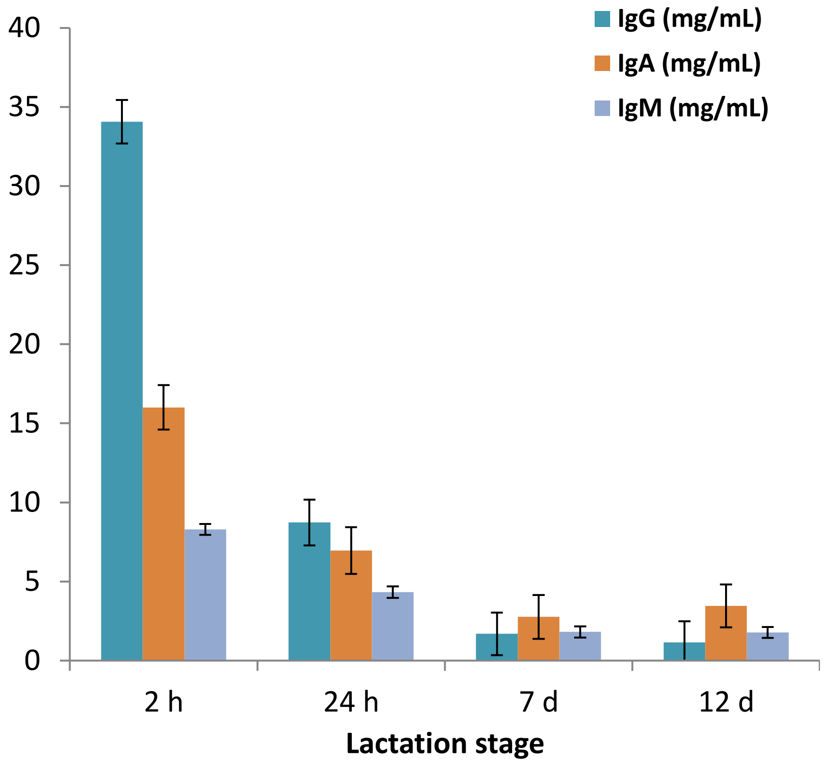
Figure 2. Illustrations of a normal (left) and a growth-restricted piglet (right). Criteria for growth restriction were 1) steep, dolphin-like forehead, 2) bulging eyes, and 3) wrinkles perpendicular to the mouth. IUGR = intrauterine growth restriction. See online version for figure in color.

Term	Criteria	Scoring	LBW	NBW
IUGR[1]	3	3	0	0
Light IUGR	1-2	2	28	9
Normal	0	1	17	36
IUGR not recorded			3	3
Total number			48	48

Hales et al 2013

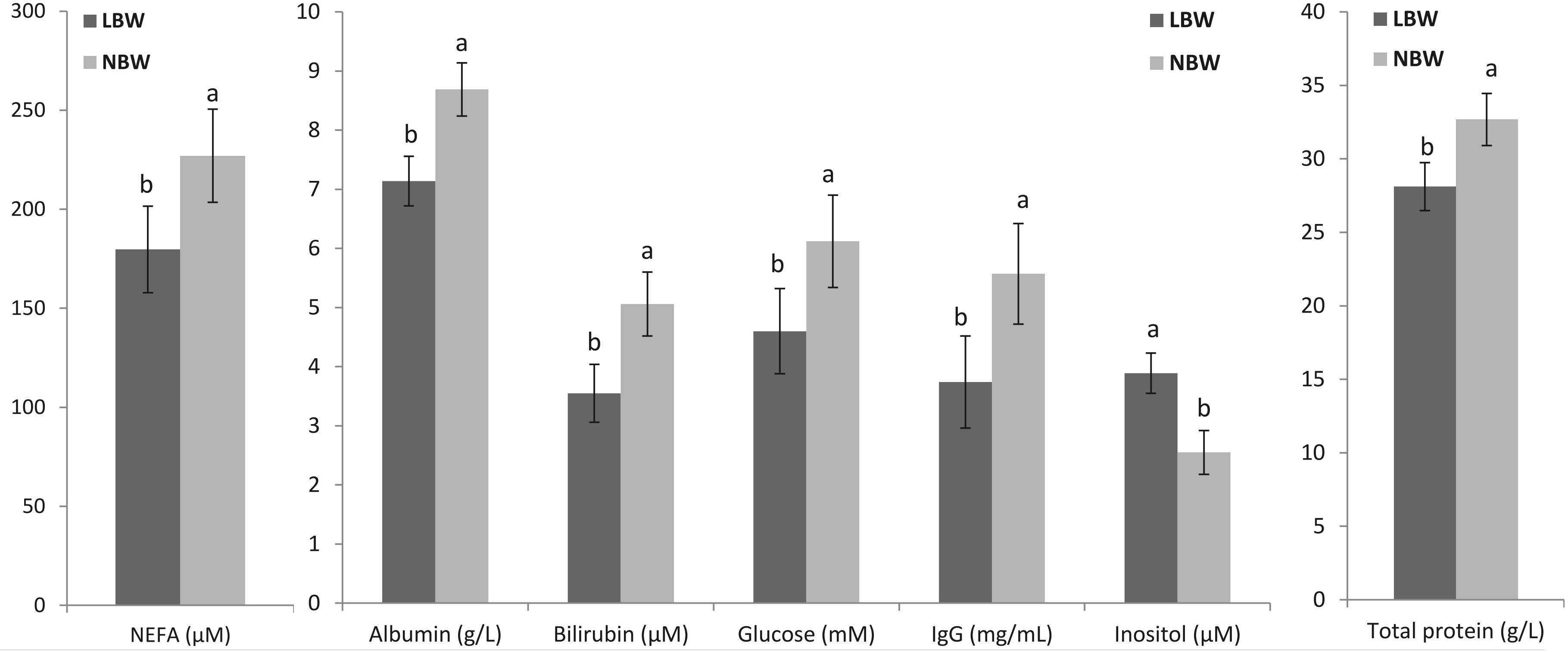
Milk proximate composition and immunoglobulins





n = 8 / lactation stage

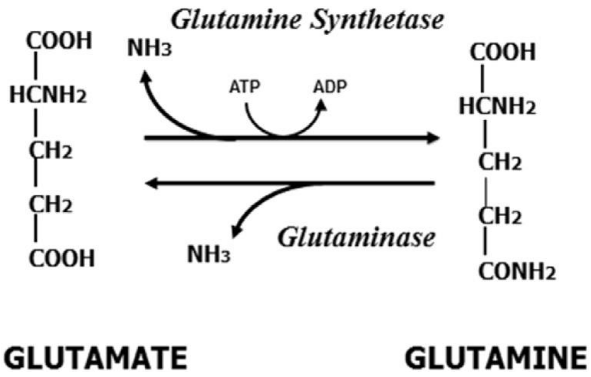
Plasma metabolites – LBW vs. NBW at 4 h

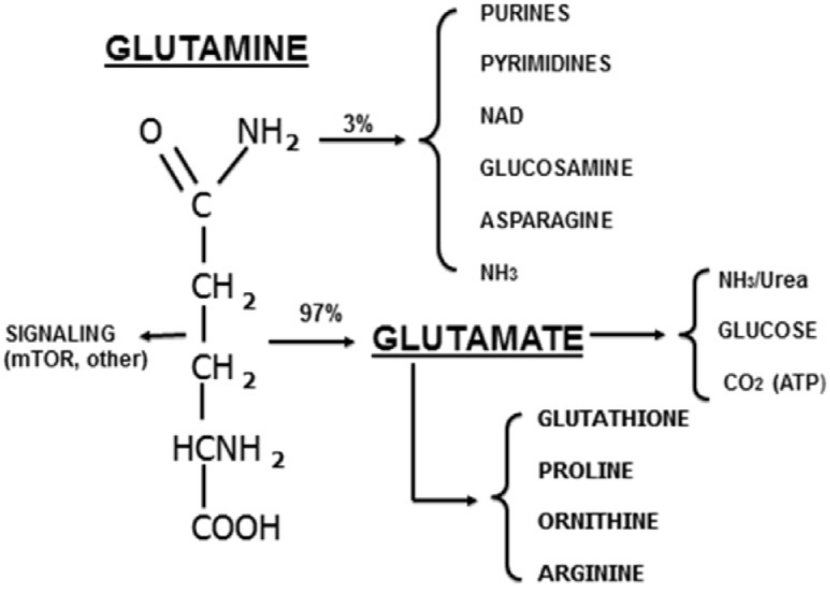


n = 35 / LBW, *n* = 36 / NBW
[a-b] Labeled columns without a common letter differ, $P < 0.05$

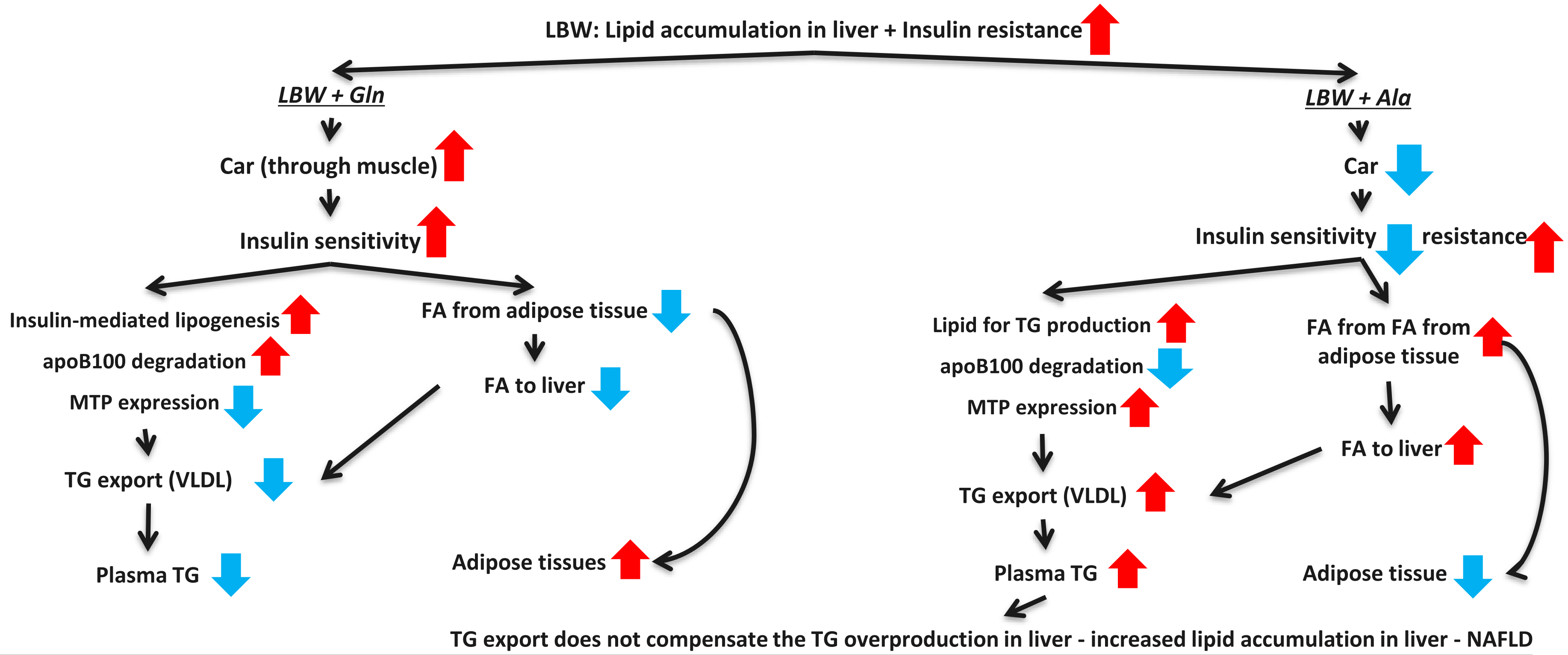
Why not Glutamate

- Our study was aimed to investigate the effect of Gln;
- Gln is an important signaling molecule, often acting by activation of (mTOR), stimulating protein synthesis, cell growth and differentiation, inhibiting protein degradation and apoptosis;
- The maintenance of Glu and Gln homeostasis is important.



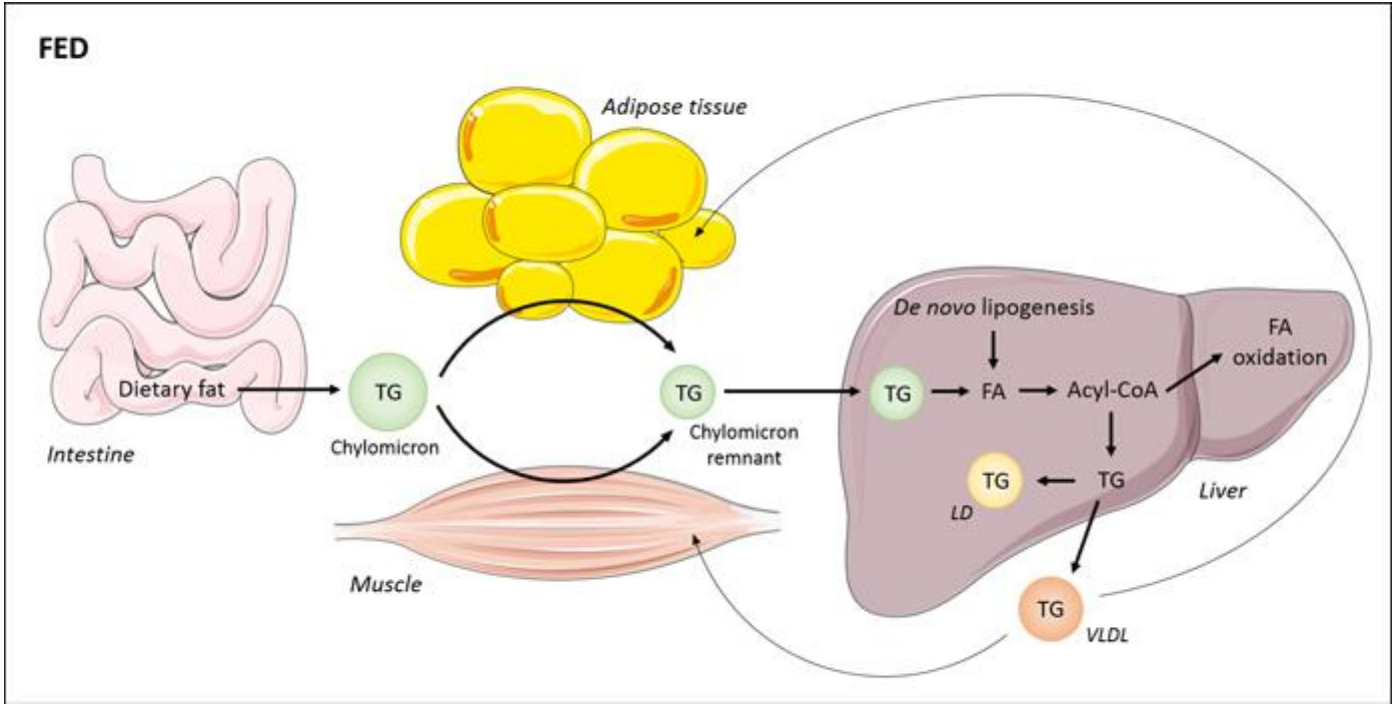


How did Gln normalize plasma TG level? – a possible mechanism



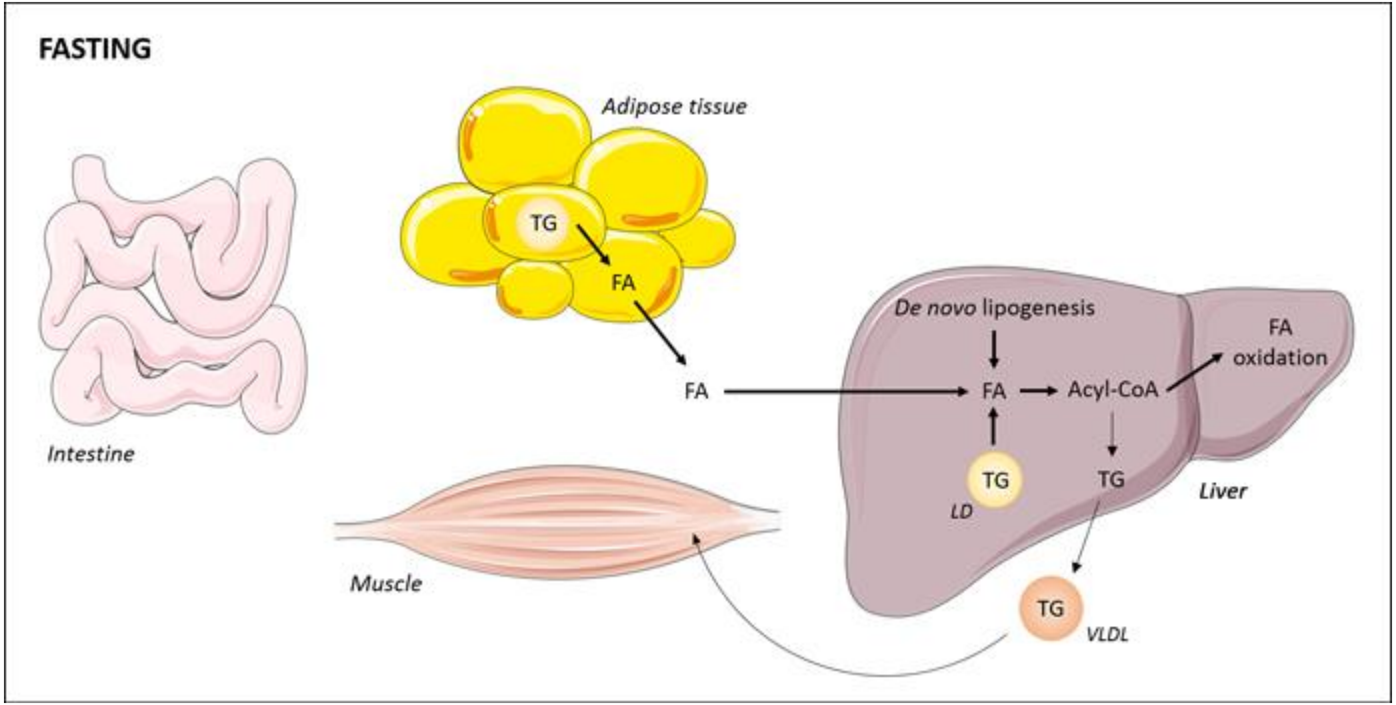
Li et al 2012, Lee et al 2015, Brown et al 2014, Mong et al 2011, Doğru-Abbasoğlu et al 2014, de Courten et al 2016, Jou et al 2008, Kawano et 2013

Lipid metabolism-fed



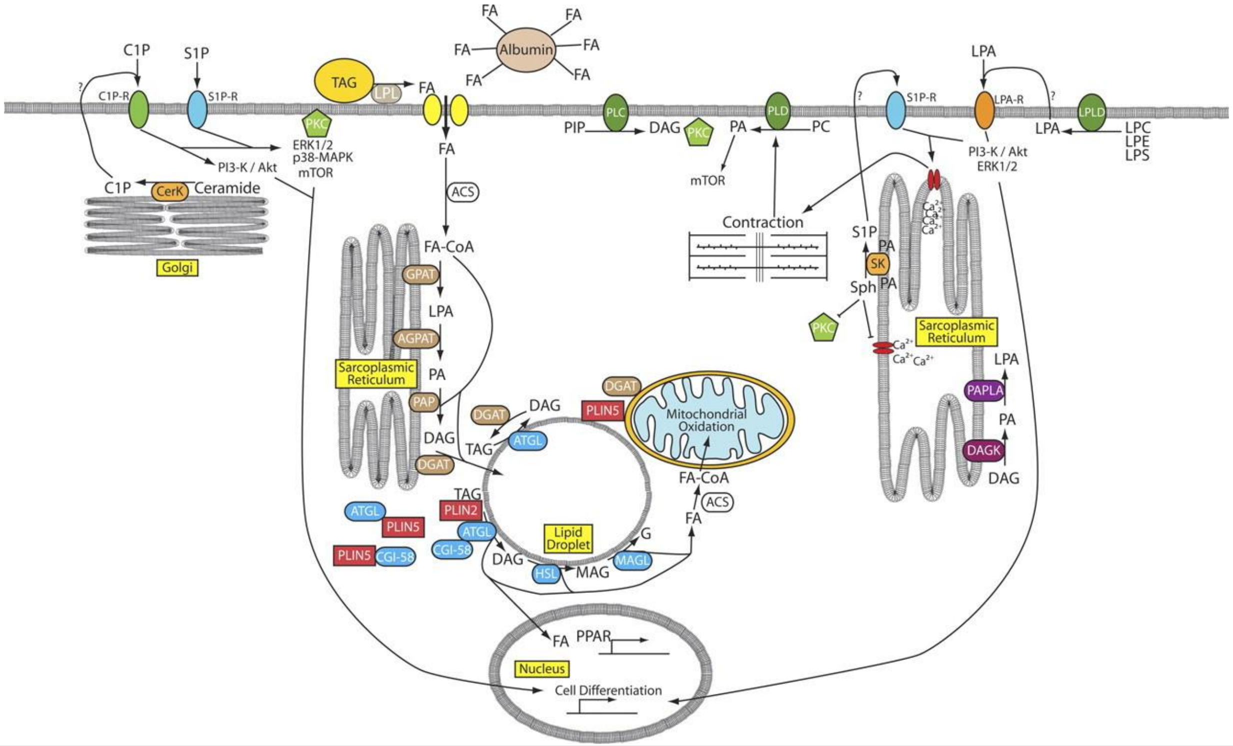
Alves-Bezerra M et al 2011

Lipid metabolism-fasting



Alves-Bezerra M et al 2011

Lipid metabolism in skeletal muscle



Watt MJ et al 2011

The alteration in gastrointestinal tract of LBW

- ❑ Reduced wall thickness
- ❑ Smaller villus length and villus area
- ❑ Weaker mucosal immunity
- ❑ Reduced barrier function

Dong et al 2014, Morise et al 2008, Metges et al 2010, Wang et al 2015, Beaulieu et al 2010, Rehfeldt et al 2006, Gondret et al 2005, 2006

The weaker mucosal immunity and reduced barrier function in LBW

- Lower absolute immune organ weight
- Decreased relative weights of the thymus, spleen, mesenteric lymph node
- Smaller number of epithelial goblet cells and lymphocytes, reduced levels of the cytokines TNF-α and IFN-γ
- Decreased gene expression of cytokines

- Higher intestinal permeability to macromolecules, suggesting an impaired barrier function, which may be a consequence of the reduced abundance of tight junction proteins in LBW (markers: FD4 and HRP for measuring paracellular and paracellular/transcellular pathways, respectively)

Dong et al 2014; Wang et al 2015

D_2O method for measuring milk intake

- Piglets received an *i.p.* injection of D_2O (0.2 mL/kg BW, 70 atom % D diluted to 20% (wt:wt) in saline) on 11 d (24 h before euthanasia)
- Following injection, piglets were placed for 1 h in an isolation box (placed in the dam's block) to prevent suckling and ensure the D_2O had equilibrated with the body water pool (with a non-experimental littermate to minimize stress)
- Blood samples were taken 1 h after injection and after being euthanized (24 h later)
- A basal blood sample from the non-experimental littermate piglet was collected to determine the background level of D enrichment
- Measurements were performed by gas isotope ratio mass spectrometry

Theil et al 2014

END