



Impact of dietary L-arginine supply during early gestation on myofiber development in newborn pigs exposed to intra-uterine crowding

Johannes Gulmann Madsen^{1,2}, Michael Kreuzer² and Giuseppe Bee¹

- ¹ Agroscope Posieux, 1725 Posieux, Switzerland
- ² ETH Zurich, Institute of Agricultural Sciences, 8092 Zurich, Switzerland

29.09.2016

Contents

- Background
- Materials and Methods
- Results
 - Reproductive traits
 - Offspring traits
 - Absolute muscle weight
 - Myofiber characteristics
 - Expression of myofiber related genes
- Conclusion
- Perspectives

Background

Selection for high prolificacy in modern sows

- Marked increase in litter size
 - More pigs suffered from intra-uterine growth retardation (IUGR)

More low birth weight (L-BtW) pigs with low viability

Background

Lower prenatal hyperplasia

Impaired postnatal growth and lean meat deposition rate



Nutritonal intervention during pregnancy?

Impact independent of BtW?

Background

Use of natural and artificially crodwed sows (surgical procedure)



Study objective

Investigate effects of dietary L-arginine supplementation in early gestation on myofiber development in the offspring at birth



L-arginine:

- Semi-essential amino acid
- Substrate for nitric oxide and polyamine synthesis
- Regulators of angiogenesis and placental growth.



L-arginine positively influences hyperplasia of primary (P) fibers in 75-d old fetuses from sows fed L-arginine from d 14-28 of gestation (Bérard et al., 2010)

Material and methods

- Experimental sow model
 - Moderately crowded intra-uterine environment (IN)
 - Use of hyperprolific sows

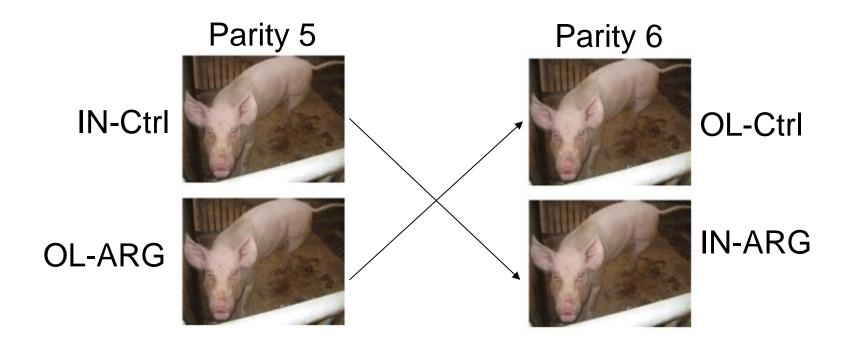
- Uncrowded intra-uterine environment (OL)
 - Use of sows subjected to unilateral oviduct ligation

O

Materials and methods

5 IN and 5 OL sows received daily from d 14 to 28 of gestation either

- 25 g L-arginine-HCl (ARG)
- 43 L-alanine (Ctrl)



O

Materials and methods

- Sows were fed daily 2.8 kg of a standard gestation diet
 - 15% CP, 7% fat, 13.70 DE MJ/kg DM.
- Recorded litter characteristics were:
 - Total born
 - Born alive
 - Birth weight
- Within litter one female and male piglet with the lowest and medium birth weight were sacrificed for further analysis.
- Traits assessed in the selected piglets:
 - Birth weight
 - Weight of the semitendinosus (STM) and psoas major (PM) muscle
 - Myofiber related traits assessed in the STM
 - Expression of myogenic-related genes analyzed in the STM



Reproductive traits

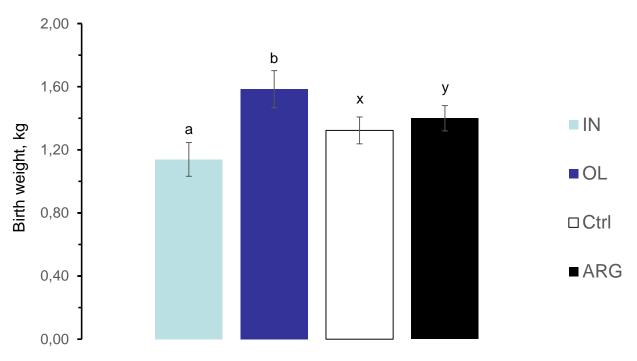
S: Surgical treatment
D: Dietary treatment

	Surgical t	reatment	Die	t		<i>P</i> -value		
Item	IN OL		Ctrl ARG		SEM	S	D	
Total born	15.4	11.3	12.9	13.6	1.21	ns	ns	
Born alive	13.8	9.7	11.1	12.4	1.07	ns	ns	
Stillborn	1.6	1.6	1.9	1.2	0.37	ns	ns	
Male:female ratio	0.55	0.46	0.48	0.52	0.056	ns	ns	
Birth weight, kg								
Total born	1.351	1.541	1.396	1.496	0.059	ns	ns	
Born alive	1.372	1.562	1.417	1.516	0.059	ns	ns	

IN vs. OL: Intra-uterine crowding had no significant effect on reproductive traits

ARG vs. Ctrl: Arginine had no significant effect on reproductive traits

Birth weight of selected pigs

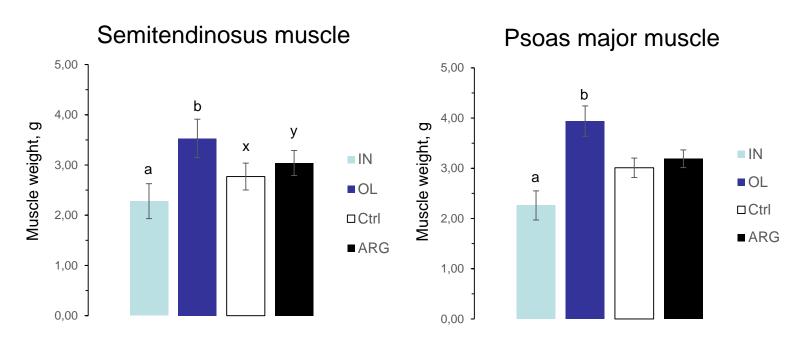


a,b LSM without a common superscript differ (P < 0.05)x,y LSM without a common superscript differ $(0.05 \le P < 0.10)$

IN vs. OL: Intra-uterine crowding lowered birth weight of selected pigs

ARG vs. Ctrl: Arginine tended to increase birth weight of selected pigs

Offspring traits: Muscle weight



^{a,b} LSM without a common superscript differ (*P* < 0.05)

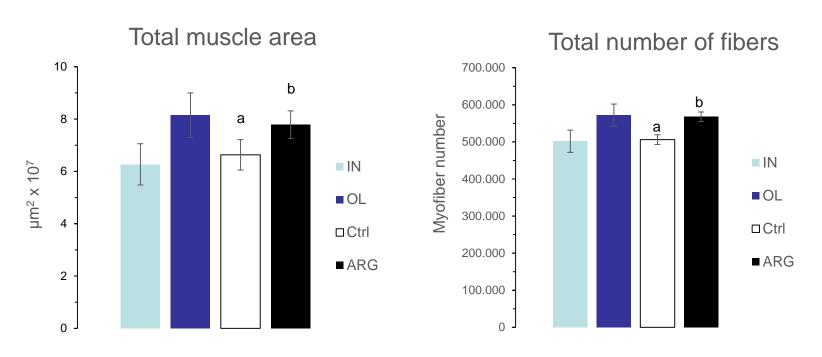
IN vs. OL: Intra-uterine crowding reduced absolute STM and PM weight

ARG vs. Ctrl: Arginine tended to increase STM but not PM weight

11

x,y LSM without a common superscript differ $(0.05 \le P < 0.10)$

Myofiber characteristics



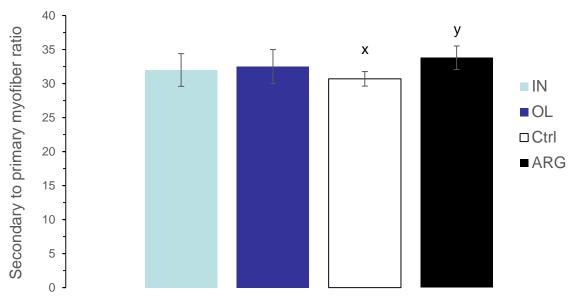
a,b LSM without a common superscript differ (P < 0.05)

IN vs. OL: Intra-uterine crowding had no effect on total muscle area and total myofiber number

ARG vs. Ctrl: Arginine increased muscle area and myofiber number

Myofiber characteristics



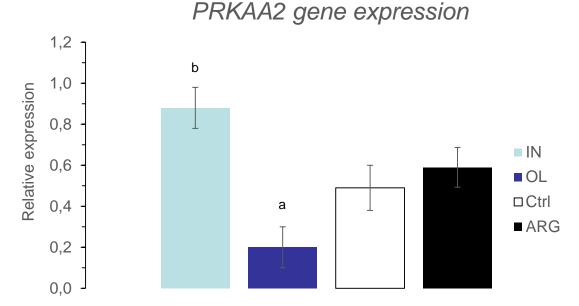


x,y LSM without a common superscript differ $(0.05 \le P < 0.10)$

IN vs. OL: Intra-uterine crowding had no effect on secondary to primary myofiber ratio

ARG vs. Ctrl: Arginine tended to increase the secondary to primary myofiber ratio

PRKAA2 gene expression



a,b LSM without a common superscript differ (P < 0.05)

Protein kinase AMP-activated catalytic subunit alpha 2 inhibitor of muscle protein synthesis

IN vs. OL: Intra-uterine crowding increased PRKAA2 gene expression

ARG vs. Ctrl: Arginine had no effect on PRKAA2 gene expression

Conclusion

- Crowding critically affects offspring's phenotype
- Early gestational supplementation of L-arginine reduces negative impact of intra-uterine crowding by increasing:
 - Birth weight of lowest and medium birth weight piglets
 - Muscle area
 - Myofiber hyperplasia
- Effect of diet is independent of the extent of crowding

Perspectives

- Further questions are: Does myofiber hyperplasia have positive effect on...
- viability and growth of L-BtW pigs in the nursery phase?
 - growth data of pigs during the nursery phase is available, but has yet to be analyzed.
- growth potential of L-BtW pigs?
 - improve weight gain in the weaning, grower and finisher phase



Thank you for your interest!



	Sow		D	iet				P-value ²		
Item	IN	OL	Ctrl	ARG	SEM -	S	D	BtW	$S \times D$	D × BtW
Birth weight	1.139	1.584	1.323	1.400	0.0992	0.008	0.068	0.564	0.137	< 0.001
Absolute muse	cle and org	gan weights ((g)							
Semitendinosus muscle	2.28	3.53	2.77	3.04	0.318	0.025	0.074	0.571	0.426	0.009
Psoas major muscle	2.26	3.94	3.01	3.19	0.247	0.001	0.232	0.543	0.891	0.024
Heart	9.44	7.87	8.34	8.96	1.382	0.418	0.159	0.806	0.005	0.032
Liver	24.15	46.77	33.44	37.47	4.417	0.004	0.053	0.529	0.290	0.022
Spleen	1.02	1.48	1.25	1.25	0.118	0.063	0.938	0.918	0.537	0.052
Lung	17.36	19.26	18.17	18.45	2.037	0.576	0.750	0.459	0.152	0.010
Kidney	8.30	12.75	10.22	10.83	1.131	0.033	0.301	0.771	0.322	0.022
Brain	32.61	34.08	33.03	33.66	0.544	0.235	0.119	0.621	0.699	0.031
Brain:liver weight ratio	1.24	0.93	1.15	1.02	0.172	0.270	0.077	0.151	0.182	0.001
Re	lative mus	cle and orga	n weights (g	/100 g <u>BtW</u>)					
Semitendinosus muscle	2.08	2.24	2.14	2.18	0.064	0.328	0.415	0.716	0.341	0.511
Psoas major muscle	2.10	2.35	2.18	2.27	0.093	0.277	0.276	0.314	0.987	0.483

26

Heart	0.68	0.66	0.69	0.66	0.015	0.492	0.030	0.770	0.720	0.338
Liver	2.34	2.77	2.49	2.62	0.125	0.128	0.192	0.629	0.142	0.343
Spleen	0.093	0.088	0.093	0.088	0.0038	0.665	0.176	0.734	0.018	0.556
Lung	1.43	1.29	1.39	1.33	0.078	0.363	0.250	0.235	0.762	0.655
Kidney	0.73	0.81	0.77	0.77	0.029	0.270	0.764	0.613	0.396	0.627
Brain	2.70	2.50	2.67	2.53	0.251	0.616	0.169	0.204	0.114	< 0.001

Results are presented as least squares means and pooled SEM.
 Probability values for the effects of surgical treatment (S), dietary treatment (D), birth weight (BtW), and interactions S × D and D × BtW.



	Sow		Ι	Diet		P-value ²					
Item	IN	OL	Ctrl	ARG	SEM _	S	D	BtW	$S \times D$	D × BtW	
Muscle area, μm² x 10 ⁷											
Total	6.270	8.150	6.63	3 7.787	0.7006	0.144	0.003	0.653	0.738	0.053	
Dark portion	2.318	3.172	2.56	5 2.925	2.0161	0.078	0.025	0.233	0.007	0.573	
Light portion	3.634	5.292	4.02	8 4.897	0.6138	0.143	0.011	0.910	0.786	0.061	
Myofiber number, N											
Total muscle	501868	572189	506238	567819	23737.8	0.236	0.003	0.136	0.604	0.475	
Dark portion											
Total myofibers	196091	201597	189259	208429	15306.2	0.884	0.131	0.002	0.231	0.560	
Primary myofibers	5972	6012	5986	5998	427.0	0.970	0.974	0.069	0.422	0.575	
Secondary myofibers	190118	195585	183272	202431	15011.1	0.883	0.124	0.002	0.231	0.559	
S:P ratio	32.0	32.5	30.7	33.8	1.91	0.912	0.051	0.356	0.643	0.337	
Light portion	305777	370592	316978	359390	21818.7	0.234	0.022	0.889	0.778	0.236	

19



	Sc	Sow		Diet		P -value 2						
Gene ³	IN	OL	Ctrl	ARG	SEM	S	D	<u>BtW</u>	$S \times D$	D × BtW		
IGF2	1.95	1.30	1.63	1.62	0.381	0.487	0.967	0.965	0.013	0.950		
IGFBP5	5.64	3.47	3.97	5.13	1.693	0.489	0.233	0.275	0.290	0.297		
MSTN ⁴	1.62	0.67	0.86	1.26	0.166	0.351	0.232	0.105	0.241	0.110		
MYF5 ⁴	0.38	0.26	0.29	0.34	0.143	0.631	0.531	0.895	0.974	0.159		
MYF6	0.62	1.08	0.88	0.82	0.391	0.623	0.859	0.893	0.664	0.631		
MYOD1 ⁴	2.08	0.57	1.27	0.94	0.250	0.223	0.340	0.056	0.885	0.418		
MYOG	0.82	1.21	1.13	0.90	0.169	0.333	0.168	0.392	0.915	0.417		
PRKAA2	0.88	0.20	0.49	0.59	0.141	0.024	0.264	0.298	0.034	0.091		