Dietary L-arginine supply during early gestation promotes myofiber hyperplasia

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Known relationships

- Litter size is negatively correlated with average litter birth weight
- Birth weight is negatively correlated with
  - survival rate in the first week after birth
  - postnatal growth
  - carcass leanness
  - some meat quality traits

![Graph showing the relationship between litter size and average birth weight](image)
Total myofiber number (TFN)

ADG vs. TFN

\[ r = 0.42 \]

G/F vs. TFN

\[ r = 0.42 \]
• In early pregnancy, arginine and ornithine level elevated in porcine amniotic and allantoic fluid

• Associated with a high syntheses rate of nitric oxide and polyamine in the porcine placenta

• Key role in angiogenesis = placental and embryonic development
• Improved placental-fetal blood flow = improved fetal development

• Increased litter size without impact of birth weight

• Impact on muscle development?
Dietary manipulation of myofiber development

Average fetal weight (d 75 of gestation)

Semitendinosus muscle

Rhomboideus muscle

S/P ratio

CON
ARG

Animal (2010), 4:10, pp 1680–1687
ARGININE ↔ MYOGENESIS

- Improved placental-fetal blood flow = improved fetal development
- Increased litter size without impact of birth weight

**Primary fibers serve as a scaffold for the formation of secondary fibers**

**Greater number primary fibers = greater number of secondary fibers**

Wu et al. 2006

Bérard and Bee 2010
Hypothesis

Based on the association between dietary arginine supply, the extent of placental vascularization, the fetal nutrient supply and muscle development 2 working hypothesis were formulated:

• Hypothesis 1:
  • Supplementing L-arginine to an early gestational diet of the dams would promote hyperplasia leading to an increased number of myofibers in their offspring at birth.

• Hypothesis 2:
  • L-arginine is especially efficient in piglets suffering from IUGR.
Experimental design

Animals:
• Intact sows (I; litter size: > 15; naturally crowded)

• OL sows (OL; unilaterally oviduct ligated; uncrowded)

All sows (n = 10) were at the beginning of the experiment in their fifth parity

Diets:
• Control (C; 100 g/d alanine from d 14 to 28 of gestation)

• Arginine (Arg; 25 g/d arginine from d 14 to 28 of gestation)
Selection criteria at farrowing

from each litter
(n = 10 per parity)

female
(n = 2)

lightest
(n = 1)

medium
(n = 1)

male
(n = 2)

lightest
(n = 1)

medium
(n = 1)
Data and sample collection and analysis

Collection of data and samples at birth
- Litter size
- BtW of all piglets born

From the selected newborn piglets
- Weight of the heart, kidney, liver, lung and spleen
- Weight of the brain
- Weight of the semitendinosus and psoas major

Histological analyses in the semitendinosus
(mATPase staining after pre-incubation at pH 4.5 and 10.2)
- CSA
- Number of P- and S-fibers
- S/P ratio
- TNF
Transcript expression of
• myogenic factor 5 (MYF5)
• myogenic differentiation factor (MYOD)
• myogenin (MYOG)
• muscle-specific regulatory factor 4 (MRF4)
• myostatin (MSTN)
• AMP-activated protein kinase catalytic subunit alpha-2 (PRKAA2)
• insulin growth factor 2 (IGF2)
• insulin growth factor binding protein 5 (IGFBP5)
## Results

### Litter characteristics

<table>
<thead>
<tr>
<th>Trait</th>
<th>Crowded C</th>
<th>Crowded Arg</th>
<th>Uncrowded C</th>
<th>Uncrowded Arg</th>
<th>SEM</th>
<th>IUC</th>
<th>DIET</th>
<th>IUC x DIET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter size, n</td>
<td>15.5</td>
<td>15.3</td>
<td>10.5</td>
<td>11.9</td>
<td>1.71</td>
<td>0.26</td>
<td>0.72</td>
<td>0.66</td>
</tr>
<tr>
<td>Total born</td>
<td>13.1</td>
<td>14.5</td>
<td>9.1</td>
<td>10.3</td>
<td>1.49</td>
<td>0.23</td>
<td>0.46</td>
<td>0.96</td>
</tr>
<tr>
<td>Born alive</td>
<td>1.22</td>
<td>1.48</td>
<td>1.57</td>
<td>1.51</td>
<td>0.068</td>
<td>0.28</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>1.24</td>
<td>1.50</td>
<td>1.59</td>
<td>1.53</td>
<td>0.068</td>
<td>0.28</td>
<td>0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>STD</td>
<td>0.24</td>
<td>0.25</td>
<td>0.18</td>
<td>0.14</td>
<td>0.056</td>
<td>0.41</td>
<td>0.87</td>
<td>0.63</td>
</tr>
</tbody>
</table>
## Results

### Birth weight of selected piglets

**DIET**, $P = 0.07$

**IUC**, $P < 0.01$

**DIET x IUC** $P = 0.14$

<table>
<thead>
<tr>
<th></th>
<th>Crowded</th>
<th>Uncrowded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>C</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>kg</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>
## Results

### Morphometric measures

<table>
<thead>
<tr>
<th>Trait expressed in g/100 g birth weight</th>
<th>Crowded C</th>
<th>Arg</th>
<th>Uncrowded C</th>
<th>Arg</th>
<th>SEM</th>
<th>IUC</th>
<th>DIET</th>
<th>IUC x DIET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.70</td>
<td>0.67</td>
<td>0.67</td>
<td>0.65</td>
<td>0.02</td>
<td>0.49</td>
<td>0.03</td>
<td>0.72</td>
</tr>
<tr>
<td>Liver</td>
<td>2.21</td>
<td>2.47</td>
<td>2.78</td>
<td>2.77</td>
<td>0.18</td>
<td>0.13</td>
<td>0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.67</td>
<td>0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Lung</td>
<td>1.47</td>
<td>1.39</td>
<td>1.31</td>
<td>1.27</td>
<td>0.11</td>
<td>0.36</td>
<td>0.25</td>
<td>0.76</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.74</td>
<td>0.72</td>
<td>0.80</td>
<td>0.82</td>
<td>0.04</td>
<td>0.27</td>
<td>0.76</td>
<td>0.40</td>
</tr>
<tr>
<td>Brain</td>
<td>2.87</td>
<td>2.53</td>
<td>2.47</td>
<td>2.50</td>
<td>0.32</td>
<td>0.62</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Brain: liver weight ratio</td>
<td>1.36</td>
<td>1.12</td>
<td>0.94</td>
<td>0.92</td>
<td>0.22</td>
<td>0.27</td>
<td>0.08</td>
<td>0.18</td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>2.09</td>
<td>2.08</td>
<td>2.19</td>
<td>2.28</td>
<td>0.11</td>
<td>0.33</td>
<td>0.42</td>
<td>0.34</td>
</tr>
<tr>
<td>Psoas major</td>
<td>2.06</td>
<td>2.14</td>
<td>2.31</td>
<td>2.39</td>
<td>0.14</td>
<td>0.28</td>
<td>0.28</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Results

Muscle area

**Crowded** Uncrowded

$m^2 \times 10^{-7}$

**dark portion**

- C
- Arg

**light portion**

- C
- Arg

**whole muscle**

- C
- Arg

Arginine supplementation increased CSA ONLY in an uncrowded environment

Arginine supplementation resulted in larger CSA

OVERALL arginine supplementation increased CSA of the ST

**Results**

Muscle area

- DIET, $P < 0.01$
- IUC, $P = 0.08$
- DIET x IUC $P < 0.01$

- DIET, $P < 0.01$
- IUC, $P = 0.24$
- DIET x IUC $P = 0.60$

- DIET, $P < 0.01$
- IUC, $P = 0.14$
- DIET x IUC $P = 0.74$
**Results**

**Myofiber number**

**dark portion**

- **C**<br>- **Arg**

ARGinine had no impact on myofiber number

**light portion**

- **C**<br>- **Arg**

ARGinine supplementation increased myofiber number

**whole muscle**

- **C**<br>- **Arg**

OVERALL ARGinine increased myofiber number

<table>
<thead>
<tr>
<th>Condition</th>
<th>Myofiber Number</th>
<th>DIET, P</th>
<th>IUC, P</th>
<th>DIET x IUC, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crowded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncrowded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td>&gt; 0.01</td>
<td>&gt; 0.24</td>
<td>&gt; 0.23</td>
</tr>
<tr>
<td><strong>Arg</strong></td>
<td></td>
<td>&lt; 0.02</td>
<td>&lt; 0.23</td>
<td>&lt; 0.78</td>
</tr>
</tbody>
</table>

**Notes:**

- DIET, P = 0.13
- IUC, P = 0.88
- DIET x IUC, P = 0.23
- DIET, P = 0.02
- IUC, P = 0.23
- DIET x IUC, P = 0.78
- DIET, P < 0.01
- IUC, P = 0.24
- DIET x IUC, P = 0.23
## Results

### Gene expression of myogenensis-related genes

<table>
<thead>
<tr>
<th>Trait</th>
<th>Crowded C</th>
<th>Crowded Arg</th>
<th>Uncrowded C</th>
<th>Uncrowded Arg</th>
<th>SEM</th>
<th>IUC</th>
<th>DIET</th>
<th>IUC × DIET</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF2</td>
<td>2.36</td>
<td>1.55</td>
<td>0.91</td>
<td>1.70</td>
<td>0.555</td>
<td>0.487</td>
<td>0.967</td>
<td>0.950</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>5.63</td>
<td>5.65</td>
<td>2.32</td>
<td>4.62</td>
<td>2.190</td>
<td>0.489</td>
<td>0.233</td>
<td>0.297</td>
</tr>
<tr>
<td>MSTN</td>
<td>1.60</td>
<td>1.64</td>
<td>0.46</td>
<td>0.98</td>
<td>1.754</td>
<td>0.351</td>
<td>0.232</td>
<td>0.110</td>
</tr>
<tr>
<td>MYF5</td>
<td>0.35</td>
<td>0.42</td>
<td>0.24</td>
<td>0.28</td>
<td>1.694</td>
<td>0.631</td>
<td>0.531</td>
<td>0.159</td>
</tr>
<tr>
<td>MYF6</td>
<td>0.58</td>
<td>0.65</td>
<td>1.18</td>
<td>0.98</td>
<td>0.576</td>
<td>0.623</td>
<td>0.859</td>
<td>0.631</td>
</tr>
<tr>
<td>MYOD1</td>
<td>2.36</td>
<td>1.84</td>
<td>0.68</td>
<td>0.48</td>
<td>2.143</td>
<td>0.223</td>
<td>0.340</td>
<td>0.418</td>
</tr>
<tr>
<td>MYOG</td>
<td>0.94</td>
<td>0.70</td>
<td>1.32</td>
<td>1.11</td>
<td>0.255</td>
<td>0.333</td>
<td>0.168</td>
<td>0.417</td>
</tr>
<tr>
<td>PRKAA2</td>
<td>0.72\textsuperscript{b}</td>
<td>1.04\textsuperscript{b}</td>
<td>0.26\textsuperscript{a}</td>
<td>0.15\textsuperscript{a}</td>
<td>0.194</td>
<td>0.024</td>
<td>0.264</td>
<td>0.091</td>
</tr>
</tbody>
</table>

\textit{PRKAA2} (inhibitor of muscle protein synthesis) greater expression related to
- lower BtW
- lower muscle weight
Supplementing L-arginine early in gestation
• reduces the negative impacts of IUGR,
  • increased hyperplasia, birth weight and STM area.

• as muscle area increased more than TNF → prenatal myofiber hypertrophy

• not especially efficient in L-BtW piglets