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

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Abstract

Background: Intra-uterine crowding (IUC) observed in hyperprolific sows impairs myofiber hyperplasia and overall fetal growth. Arginine supplementation (ARG) in gestation diets has been shown to positively affect litter and muscle development. The study objective was to assess whether the effect of ARG on offspring characteristics, with special emphasis on myofiber hyperplasia, differs under IUC conditions from these responses, because in that situation growth retardation is particularly prevalent due to reduced fetal nutrient supply. Unilateral oviduct ligation (OL) was used as a model for an uncrowded and hyperprolificacy (IN) as a model for a crowded intra-uterine environment.

Methods: Five OL and five IN sows were fed a diet supplemented daily with either 43 g L-alanine (Ctrl) or 25 g L-arginine from d 14 to 28 of gestation in a cross-over design (two periods: 5th and 6th parity). At farrowing, two male and two female offspring, respectively, with a low and intermediate birth weight (BtW) were selected. After euthanization, the Semitendinosus muscle (STM) was removed and weighed, and the light and dark portions (STMd and STMl) were prepared for myofiber histochemistry using ATPase staining and the entire STM for gene expression analysis of myogenesis-related genes using RT-qPCR. In addition, various organs were removed and weighed. Data were analyzed using the MIXED model in SYSTAT.

Results: No effect of either IUC or dietary treatment was found in litter characteristics. Offspring of ARG sows displayed a greater muscle area in STM (P < 0.01) as a result of the greater myofiber hyperplasia (P < 0.01). The increase was more distinct in the STMd (P < 0.05) than in the STMl (P = 0.131). Offspring of OL sows were heavier at birth (P < 0.01), had a heavier STM (P < 0.05), liver (P < 0.01) and kidney (P < 0.05), but when expressed relative to birth weight, these differences were absent. In addition, IUC had an effect (P < 0.05) on the expression of one of the myogenesis-related genes investigated.

Conclusions: Independent from the extent of IUC, ARG improved BtW, muscle and organ weights and myofiber hyperplasia in offspring.

Keywords: Dietary supplement, Early gestation, Intra-uterine growth restriction, Myofiber hyperplasia, Neonate, Sow prolificacy

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Background
In the last decade, the selection for high prolificacy in modern sow herds has led to a marked increase in litter sizes. One consistent outcome of this strategy was the increasing number of less vital and less mature low birth weight (L-BtW) piglets [1, 2]. In these piglets, prenatal muscle development is impaired [3, 4] as evidenced by the lower myofiber number and the greater number of myofibers still expressing the fetal myosin heavy chain isoform at birth [4]. Compared with their heavier siblings, the lower prenatal myofiber hyperplasia observed in underprivileged piglets has negative consequences on postnatal growth efficiency [5] and lean meat deposition rate [6, 7].

The pig muscle develops in a biphasic manner [8] (Fig. 1). In a first wave from d 35-55 of gestation, an initial population of myoblasts fuse to form primary (P) myofibers. From d 55-90 of gestation, these primary myofibers serve as scaffold for the fusion of a second larger population of myoblasts, the so-called secondary (S) myofibers [9, 10]. As opposed to S myofiber development where crowding of the uterus at early gestation appears to be a compromising factor [11], P myofiber number is assumed to be a fixed genetic component, and its development is assumed to be unaffected by conditions occurring in utero [12]. However, a recent study showed that hyperplasia of P myofibers was greater in the Semitendinosus muscle (STM) of 75-day-old fetuses originating from sows fed a diet supplemented with l-arginine from d 14-28 of gestation [13]. Because supplementation of l-arginine occurred before the start of P myofiber formation, the authors hypothesized that this effect was an indirect effect. Earlier supplementation of l-arginine from d 0-14 is not advisable because of its detrimental effect on embryonic survival, which is likely due to reduced progesterone secretion [14]. Arginine is a common substrate for nitric oxide and polyamine synthesis [15], both of which are key regulators of angiogenesis and placental growth [16]. Therefore, an increased dietary arginine supply might improve the fetal nutrient supply [17] and ultimately promote myofiber hyperplasia. This mode of action could be interesting especially for offspring from prolific sows suffering from intra-uterine growth retardation (IUGR) due to a crowded intra-uterine environment which impairs placental and, consequently, fetal development.

Based on the aforementioned association between dietary arginine supply, the extent of placental vascularization, the fetal nutrient supply and muscle development, it was hypothesized that 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. A second hypothesis tested the theory of whether l-arginine would be especially efficient in piglets suffering from IUGR. Two sow models were established for the present study, one ‘non-crowded’ and the other ‘relatively crowded’ simulated by intact (IN) prolific sows. The non-crowded intra-uterine environment was mimicked by using unilaterally oviduct ligated (OL) sows. In these sows, oocytes ovulated from the ovary ipsilateral to the ligated oviduct are prevented from being fertilized and entering the uterus. Therefore, the number of embryos and the extent of crowding in utero is markedly reduced in OL compared with IN sows [13, 17].

Methods
Animals and dietary treatments
The study was conducted as a 2 × 2 crossover design (two periods for each sow in their 5th and 6th parity). It involved five OL sows originating from a previous experiment of Pardo et al. [18] and five prolific IN sows.
The five IN sows were siblings to the five OL sows. Except from d 14 to 28 of gestation, the sows were reared from mating to farrowing with other multiparous sows in group pens equipped with an automatic feeder (Compendent, Model 2000, Schauer, Prambachkirchen, Austria) and were offered 2.8 kg of a standard gestation diet daily (Table 1). From d 14 to 28 of gestation, the sows were kept in individual pens. They were randomly allotted to receive daily either 25 g L-arginine HCl (ARG), an amount based on the previous findings of Bérard and Bee [13], or 43 g L-alanine (Ctrl) both as top dressing for 14 d. Alanine was used to compensate for the increased amount of nitrogen in the ARG treatment making the two dietary treatments isonitrogenous.

### Data and tissue sample collection at farrowing

At the end of farrowing, litter characteristics including the number of piglets born alive and stillborn and their individual body weights were recorded. At the day of farrowing, two female and two male piglets per sow per parity were intentionally sacrificed, one with the lowest BtW (L: average ± standard deviation (SD): 1.24 ± 0.30 kg) and one with intermediate BtW (M: average ± STD: 1.49 ± 0.26 kg). Thus, the aim was to include a total of 80 piglets in the study. However, due to unforeseen events at farrowing, the final number was only 70 piglets, which were balanced accordingly between the extent of crowding (38 vs. 32, IN and OL), dietary treatment (36 vs. 34, Ctrl and ARG), BtW (33 vs. 37, L and M), sex (34 vs. 36, male and female), and parity (33 vs. 37, 5th and 6th). Pigs with a BtW of < 800 g were considered runts and were not included in the study. The selected piglets were anaesthetized using an isoflurane-oxygen mixture [4% vol/vol] and subsequently euthanized by exsanguination. Subsequently, the complete Semitendinosus muscle (STM) and Psoas major muscle (PM) were removed and weighed. The STM was split into the light and dark portions (STMl and STMd). A section from each portion was removed from the middle of the muscle, snap-frozen in 2-methylbutane cooled in liquid nitrogen and subsequently stored at −80°C until histochemical analysis was performed. Subsequently, spleen, kidneys, heart, lungs, liver and brain were removed and weighed.

### Histochemical analysis of the semitendinosus muscle

The P and S myofibers were differentiated histochemically using the protocol described previously by Bérard et al. [4]. Briefly, 10 μm cross-sections of the STMd and STMl were prepared and stained for the determination of myofilibrillar ATPase activity after acid (pH 4.5) or alkaline (pH 10.3) preincubation. In the STMd, the P myofibers stain dark, and the S myofibers light, using the acid preincubation condition, whereas the opposite occurs after basic preincubation. This differentiation was possible in the STMd, whereas, in accordance with observations of the study by Bérard et al. [4], P and S myofibers could not be differentiated in the STMl of newborn pigs using this mATPase histochemistry assay. A sectional cut of the entire muscle area was stained using anti-slow myosin heavy chain monoclonal antibody (Novocastra lyophilized mouse monoclonal antibody myosin heavy chain (NCL-MHCs) diluted 1:20 in ultrapure water; Novocastra, Newcastle, UK). This allowed a clear determination of the STMd and STMl cross-sectional areas.

### Table 1 Ingredients and calculated nutrient composition of the gestation diet

| Item | Gestation diet |
|------|----------------|
| Item | Ingredients, % as-fed | Calculated composition, % DM |
| Barley | 25.0 | Dry matter, % of wet weight 88.35 |
| Oat | 20.0 | Total ash 7.20 |
| Dried sugar beet pulp | 14.3 | Ether extract 6.80 |
| Wheat bran | 10.0 | Crude protein 15.10 |
| Soybean meal | 5.7 | Digestible energy, MJ/kg DM 13.70 |
| Dried apple pomace | 5.6 |  |
| Dried whole maize plant | 5.0 |  |
| Animal fat (70% lard and 30 % tallow) | 3.0 |  |
| Linseed meal | 2.0 |  |
| Potato protein | 2.0 |  |
| Rapeseed meal | 2.0 |  |
| Molasses | 2.0 |  |
| Dicalcium phosphate | 1.16 |  |
| Calcium carbonate | 0.75 |  |
| Sodium chloride | 0.56 |  |
| Pellan2 | 0.40 |  |
| Vitamin-mineral premix3 | 0.40 |  |
| Amino acids4, % | 0.08 |  |
| L-lysine HCl | 0.08 |  |
| L-threonine | 0.06 |  |

1In the ARG group, the pellets were top dressed with 25 g L-arginine HCl/ (sow·d), and in the Ctrl group, the pellets were top dressed with 43 g L-alanine/(sow·d)
2Binder that aids in pellet formation (Mikro-Technik, GmbH & Co. KG, Germany)
3Contains: vitamin A: 2,000,000 U/kg; vitamin D3: 200,000 U/kg; vitamin E: 10 g/kg; f: 137.4 mg/kg; Mn: 5 g/kg; Cu: 1.75 g/kg; Zn: 1.4 g/kg; Se: 50 mg/kg
4Calculated amino acid composition, g/kg DM: alanine: 6.42; arginine: 8.51; aspartate plus asparagine: 12.70; cystine: 3.15; glutamate plus glutamine: 27.50; glycine: 6.54; histidine: 3.42; isoleucine: 5.74; leucine: 10.72; lysine: 7.90; methionine: 2.46; phenylalanine: 7.00; proline: 9.59; serine: 6.73; threonine: 6.30; tryptophan: 1.83; tyrosine: 5.03; valine: 7.38
The number of P myofibers were determined in the mATPase sections after acid pre-incubation, where 750 P myofibers were counted in an area of 0.89 mm². The number of P myofibers, counted in the selected area, and the cross-sectional area of the STM₈ was used to estimate the total number of P myofibers. The number of S myofibers was determined in mATPase sections after alkali preincubation. Four images were taken and analyzed using the analySIS software 5.0 (Soft Imagine System, Olympus Soft Imaging Solutions GmbH, Münster, Germany). At least 33 P myofibers were selected, and all the surrounding S myofibers were counted (> 1000 S myofibers). This permitted estimating the total S myofiber number as well as calculating the S:P myofiber ratio. The total number of myofibers (TNF) in the STM₈ was calculated from the respective estimated total number of P and S myofibers. In the STM₈, the number of myofibers was determined in mATPase sections after acid preincubation, where > 3000 myofibers were counted in an area of 1.47 mm². The number of counted myofibers, the according measurement area (1.47 mm²) and the cross-sectional area of the STM₈ were used to estimate TNF.

Gene expression analysis of myogensis-related genes in the Semitendinosus muscle

Of the 70 piglets, samples from five individuals (1 L-BtW and 1 M-BtW male from ARG-IN sows, 2 L-BtW females from ALA-IN sows and 1 L-BtW male from ALA-IN sow) were of too poor quality for analysis of gene expression, thus in a total of 65 piglets, total mRNA was extracted from 50 mg of ST muscle by the phenol-chloroform extraction protocol involving homogenization with IKA® T10 basic Ultra-Turrax® (IKA, Staufen, Germany) and centrifugation steps were carried out at 100 μg for 8 min. After a last centrifugation step at 7,500 × g for 8 min, the ethanol was discarded and the pellet was dried at room temperature. Finally, the mRNA was resuspended in 100 μL nuclease-free water under agitation at 55 °C. Quality and concentrations of sample mRNA were measured with a NanoDrop® ND-1000 (Thermo Scientific, Waltham, Massachusetts, USA). Primers for both target and reference genes are listed in Table 2. All primers were designed with Primer3 v.4.0.0 (http://primer3.wi.mit.edu/), tested for dimerization with OligoAnalyzer® v.3.1 (Integrated DNA Technologies, Coralville, Iowa, USA), and subsequently purchased in SePOP quality dissolved in water (Eurogentec, Seraing, Belgium). Reverse transcription of complementary DNA (cDNA) was performed with ImProm-II™ Reverse Transcription System (Promega, Dübendorf, Switzerland).

Statistical analysis

Data were analyzed using the MIXED procedure of SYSTAT version 13 (Systat Software, San Jose, CA). All data were tested for normality of residuals. For litter traits, data were analyzed including IUC and dietary treatment (DIET), IUC × DIET interaction, parity, and
boar as fixed effect, offspring as random effect, and sow as the experimental unit. Data on absolute and relative STM and PM weight, absolute and relative organ weight, total area of STM, TNF of STM, P and S number of myofibers, the S:P ratio of STM, STM l, and gene expression were analyzed including the main factors of IUC, DIET, sex, sow, parity, BtW (nested within sow), IUC × D and BtW × DIET interaction as fixed, and boar as random effect. For all analyses of the piglet data, sow was used as variable in a repeated measurement procedure. Birth weight was nested within sow as birth weight intervals (L and M) were not unique among sows, leading to the individual piglet being assigned either L or M depending on the dam. The final model was determined by performing backward elimination of non-significant three- and two-way interactions. Least squares means of the interaction means concerning the main factors IUC and DIET are presented in the result tables. As the effect of sex and BtW was rarely significant, these least square means were only reported in the text when $P < 0.05$. The PAIRWISE option using the Tukey adjustment was used to determine differences between interaction effects when two-way interactions occurred. Differences were considered statistically significant at $P < 0.05$ and as tendency at $0.05 < P < 0.10$.

Results

Table 2 Forward and reverse primers of myogenesis-related genes and reference genes

| Gene      | Accession no. | Forward primer (from 5' to 3') | Reverse primer (from 5' to 3') |
|-----------|---------------|--------------------------------|--------------------------------|
| IGF2      | NM_213883     | TGGCATCGTGGAAGAGTG              | AGGTGTCTATAGCGGAAGAC           |
| IGFBP5    | U41340        | GTGACCTGCACAAACGTGA            | AAGCTGTGGACTGGAGCT             |
| MSTN      | NM_214435     | CCCCCTACACTCTACCTACAACA        | CAATCTACTGCTCTGCAAA           |
| MYF5      | Y17154        | CTCGCTGAACACACGCCCCCTCCCCCTCT  | ATACGAGCGCCCCCTGGAAAT         |
| MYF6      | NM_001244672  | CGCCGATCACTACACCGAGGGG       | AGGTGTGGGAGGTGAGCT             |
| MYOD1     | NM_001002824  | GGTGACTAGAGGACCGACCCTCA       | ATAGGTGGCCGTGAGCT             |
| MYOG      | NM_001012406  | CAACCGAGGAGCGAGGAGGAGGAGGAGGAGGAG | GAGGTAGGAGGAGGTGAGCT |
| PRKAA2    | NM_214266     | CCCCCTACACTACCTACAACCTCC      | GACTCTACTGAGGATCTCAT           |

Reference genes

| RPL4      | DQ845176     | CAAGAGTAACCTACACCTC            | GAACCTACTGAGGATCTCAT          |
| TBP       | DQ178129     | GATGGACGCTGGGAGGGTGGAGGGGG     | AGGCACGAGCAGGAGGGCAA          |

$^{1}$IGF2 Insulin-like growth factor 2, IGFBP5 Insulin-like growth factor binding protein 5, MSTN Myostatin, MYF5 myogenic factor 5, MYF6 myogenic factor 6, MYOD1 Myogenic differentiation 1, MYOG Myogenin (myogenic factor 4), PRKAA2 Protein kinase AMP-activated, alpha 2 catalytic subunit, RPL4 (LOC100038029) Ribosomal protein L4, TBP TATA box binding protein

Birth weight and absolute and relative muscle and organ weights

There were no significant effects of sow type and diet type on BtW of total born and born alive piglets (Table 3). Birth weight of males born alive from IN sows fed the ARG diet were heavier in tendency than those fed the Ctrl diet, whereas BtW of males born alive were lighter in tendency when originating from OL sows fed the ARG instead of the Ctrl diet (IUC × DIET interaction; $P = 0.087$). The average BtW of the selected offspring was lower than of the litter average because half of the piglets were of deliberately low BtW (Table 4). The piglets selected from the OL sows compared with those from the IN sows were heavier ($P = 0.008$) at birth, and had greater (P ≤ 0.033) STM, PM, liver and kidney weights. The weight of the spleen tended ($P = 0.068$) to be greater in OL compared with IN offspring. When expressed per 100 g BtW, the relative weights of the muscles and organs did not differ. The selected offspring from ARG compared with Ctrl sows tended ($P < 0.10$) to have greater BtW, absolute STM weight, liver weight and a lower brain:liver weight ratio. Except for the relative heart weight, which was lower ($P = 0.030$) in ARG

Sow performance and litter characteristics

The number of total born and born alive piglets, and the male to female ratio were not different between IN and OL sows and were also not affected by the dietary ARG supply (Table 3). Average litter BtW, average BtW of female and male newborns as well as BtW variability, expressed as standard deviation (SD), also did not differ between IN and OL sows and between the ARG and Ctrl dietary treatments. Regarding stillborns, the five IN sows gave birth to one, two, two, four and five stillborn piglets, respectively, and only one OL sow gave birth to one stillborn piglet. Thus, the number of stillborn piglets were unevenly distributed: IN-Ctrl = 7; IN-ARG = 7; OL-Ctrl = 1; OL-ARG = 0. Due to the greatly unbalanced distribution between experimental treatment groups, statistical analysis of number and BtW of stillborn piglets was not performed.
Table 3  Reproductive characteristics of oviduct ligated (OL; non crowded) and intact (IN; relatively crowded) sows fed an unsupplemented (Ctrl) or L-arginine supplemented gestation diet (ARG) from d 14 to 28 of gestation 1

| Item                  | IN Ctrl | ARG | OL Ctrl | ARG | SEM | IUC | DIET | IUC × DIET |
|-----------------------|---------|-----|---------|-----|-----|-----|------|------------|
| Observations          | 5       | 5   | 5       | 5   |     |     |      |            |
| Total born            | 15.5    | 15.3| 10.5    | 11.9| 1.71| 0.255| 0.722| 0.657      |
| Born alive            | 13.1    | 14.5| 9.1     | 10.3| 1.49| 0.230| 0.463| 0.961      |
| Male:female ratio     | 0.47    | 0.62| 0.50    | 0.41| 0.093| 0.508| 0.732| 0.368      |
| Birth weight, kg      |         |     |         |     |     |      |      |            |
| Total born            | 1.22    | 1.48| 1.57    | 1.51| 0.068| 0.278| 0.214| 0.126      |
| Born alive            | 1.24    | 1.50| 1.59    | 1.53| 0.068| 0.277| 0.224| 0.133      |
|                       |         |     |         |     |     |      |      |            |

1 Results are presented as least squares means of interactions between the two main factors extent of intra uterine crowding and dietary treatment and pooled SEM
2 Probability values for the effects of extent of intra uterine crowding (IUC), dietary treatment (DIET), and interaction IUC × DIET
3 Measure for variability in birth weight, expressed as standard deviation (SD).

Table 4  Birth weight, absolute and relative muscle and organ weights and brain:liver weight ratio of offspring born from oviduct ligated (OL; non crowded) and intact (IN; relatively crowded) fed an unsupplemented (Ctrl) or L-arginine supplemented diet (ARG) from d 14 to 28 of gestation 1

| Item                  | IN Ctrl | ARG | OL Ctrl | ARG | SEM | IUC | DIET | IUC × DIET |
|-----------------------|---------|-----|---------|-----|-----|-----|------|------------|
| Observations          | 19      | 19  | 17      | 15  |     |     |      |            |
| Birth weight, kg      | 1.06    | 1.21| 1.58    | 1.59| 0.128| 0.008| 0.068| 0.564      |
|                       |         |     |         |     |     |      |      | < 0.001   |
| Weights, g            |         |     |         |     |     |      |      |            |
| Semitendinosus muscle | 2.08    | 2.48| 3.47    | 3.60| 0.417| 0.025| 0.074| 0.571      |
| Psoas major muscle    | 2.15    | 2.36| 3.86    | 4.02| 0.343| 0.001| 0.232| 0.543      |
| Heart                 | 8.34a   | 10.54ab| 8.35ab| 7.83ab| 1.695| 0.418| 0.159| 0.806      |
| Liver                 | 20.91   | 27.39| 45.98  | 47.55| 5.808| 0.004| 0.053| 0.529      |
| Spleen                | 1.05    | 1.00| 1.45    | 1.50| 0.164| 0.063| 0.938| 0.918      |
| Lungs                 | 16.48   | 18.24| 19.86  | 18.66| 2.655| 0.576| 0.750| 0.459      |
| Kidneys               | 7.68    | 8.93| 12.77   | 12.73| 1.516| 0.033| 0.301| 0.771      |
| Brain                 | 32.22   | 33.01| 33.85  | 34.32| 0.781| 0.235| 0.119| 0.621      |
| Brain:liver weight ratio | 1.36  | 1.12| 0.94    | 0.92| 0.222| 0.270| 0.077| 0.151      |
|                       |         |     |         |     |     |      |      | 0.182      |
| Weights, g/100 g BtW  |         |     |         |     |     |      |      |            |
| Semitendinosus muscle | 2.09    | 2.08| 2.19    | 2.28| 0.095| 0.328| 0.415| 0.716      |
| Psoas major muscle    | 2.06    | 2.14| 2.31    | 2.39| 0.140| 0.277| 0.276| 0.314      |
| Heart                 | 0.70    | 0.67| 0.67    | 0.65| 0.023| 0.402| 0.030| 0.770      |
| Liver                 | 2.21    | 2.47| 2.78    | 2.77| 0.179| 0.128| 0.192| 0.629      |
| Spleen                | 0.10a   | 0.09ab| 0.09a  | 0.09ab| 0.006| 0.665| 0.176| 0.734      |
| Lung                  | 1.47    | 1.39| 1.31    | 1.27| 0.109| 0.363| 0.250| 0.235      |
| Kidney                | 0.74    | 0.72| 0.80    | 0.82| 0.043| 0.270| 0.764| 0.613      |
| Brain                 | 2.87    | 2.53| 2.47    | 2.50| 0.323| 0.616| 0.169| 0.204      |< 0.014   |

1 Results are presented as least squares means of interactions between the two main factors extent of intra uterine crowding and dietary treatment and pooled SEM
2 Probability values for the effects of extent of intra uterine crowding (IUC), dietary treatment (DIET), birth weight (BtW), and interactions IUC × DIET and DIET × BtW
3 Within a row for the main factor treatment, least squares means without a common superscript differ (P < 0.05)
than Ctrl offspring, muscle and organ weights relative to BtW were not affected by the dietary treatment of the sow. There was an IUC × DIET interaction \((P = 0.005)\) indicating that absolute heart and relative spleen weight were greater and lower, respectively, in IN-ARG compared with IN-Ctrl piglets. Birth weight had no effect on either absolute or relative STM, PM, organ weights and brain:liver weight ratio. There were a number of significant \((P < 0.05)\) diet × BtW interactions. Compared with L-piglets, M-piglets born from sows fed the ARG, but not the Ctrl diet, had heavier STM \((L-\text{ARG} = 2.679 \text{ vs. } M-\text{ARG} = 2.547 \text{ g})\), hearts \((L-\text{ARG} = 8.387 \text{ vs. } M-\text{ARG} = 9.538 \text{ g})\), livers \((L-\text{ARG} = 33.87 \text{ vs. } M-\text{ARG} = 41.07 \text{ g})\), spleens \((L-\text{ARG} = 1.07 \text{ vs. } M-\text{ARG} = 1.42 \text{ g})\), lungs \((L-\text{ARG} = 17.00 \text{ vs. } M-\text{ARG} = 19.90 \text{ g})\) and kidneys \((L-\text{ARG} = 9.66 \text{ vs. } M-\text{ARG} = 12.00 \text{ g})\). On the other hand, L-piglets compared with M-piglets born from sows fed the ARG had higher relative brain weights \((L-\text{ARG} = 2.80 \text{ vs. } M-\text{ARG} = 2.25)\) and a higher brain:liver weight ratio \((L-\text{ARG} = 1.14 \text{ vs. } M-\text{ARG} = 0.90)\) \((\text{DIET} \times \text{BtW interaction}; \ P < 0.001)\). No such differentiation was observed between L-Ctrl vs. M-Ctrl. It is also noteworthy that the absolute brain weight did not differ between L-ARG \((33.5 \text{ g})\) and M-ARG \((33.8 \text{ g})\). Together, these results suggest that besides the obvious positive association between BtW and piglet organ size, an early gestational ARG supplementation has slightly greater effect on organ development of offspring with intermediate compared with low BtW. Finally, males and females differed \((P < 0.05)\) in absolute weights of liver \((34.18 \text{ vs. } 36.73 \text{ g})\), lung weight \((18.80 \text{ vs. } 17.82 \text{ g})\) and relative heart weight \((0.69 \text{ vs. } 0.66 \text{ g}/100 \text{ g BtW})\).

### Myofiber characteristics of the Semitendinosus muscle

Except for the greater \((P = 0.078)\) area of the STM\(_d\) in OL compared with IN offspring, the extent of IUC did not affect total muscle area, muscle area of the STM\(_d\) and STM\(_l\) myofiber number of selected offspring \((\text{Table 5})\). By contrast, the areas of the STM\(_d\) and STM\(_l\) and, consequently, of the whole STM were larger \((P \leq 0.025)\) in offspring born from ARG sows compared with Ctrl sows. However, regarding the STM\(_d\) area, ARG supplementation had a greater impact on OL compared to IN offspring \((\text{IUC} \times \text{DIET}; \ P = 0.007)\). In addition, the STM tended to be larger in M piglets from ARG sows compared with piglets from the other BtW categories \((\text{STM}; \text{M-ARG} = 8.573; \text{L-ARG} = 7.002; \text{M-Ctrl} = 6.996; \text{L-Ctrl} = 6.271 \text{ μm}^2 \times 10^3\text{, and STM}_l \text{M-ARG} = 5.494; \text{L-ARG} = 4.301; \text{M-Ctrl} = 4.181; \text{L-Ctrl} = 3.875 \text{ μm}^2 \times 10^3\); \text{DIET} \times \text{BtW interaction}; \ P \leq 0.061)\). Total myofiber number was 12% greater \((P = 0.003)\) in the ARG group; this is primarily a result of a 13% greater \((P = 0.022)\) TNF of the STM\(_l\). In the STM\(_d\), where differentiation between P and S myofibers was possible, the number of S myofibers, but not P myofibers, was numerically \((P = 0.124)\) greater in piglets of ARG sows. As a consequence, the S:P myofiber ratio tended \((P = 0.051)\) to be greater in the STM\(_d\) of these piglets. While L- and M-piglets did not differ with respect to total muscle area and TNF of the whole STM, BtW had an effect \((P < 0.05)\) on TNF in the STM\(_d\) \((L = 188,925 \text{ vs. } M = 208,792)\), the number of S myofibers \((L = 182,927 \text{ vs. } M = 202,776)\) and tended to have an effect \((P = 0.069)\) on the number of P myofibers \((L = 5,998 \text{ vs. } M = 5,986)\) also in the STM\(_d\). Due to the lack of an effect of BtW and of a DIET × BtW interaction in TNF, the positive effect of ARG supplementation on myofiber hyperplasia was similar for L and M piglets \((L = 506,274 \text{ vs. } M = 567,783 \text{ myofibers})\).

### Gene expression in the Semitendinosus muscle

The level of \(\text{PRKAA2}\) expression in the STM was greater \((P = 0.024)\) in IN compared with OL offspring \((\text{Table 6})\). In addition, ARG appeared to have a greater impact on the expression of this gene in IN than in OL offspring \((\text{IUC} \times \text{DIET}; \ P = 0.034)\). Furthermore, a IUC × DIET interaction effect \((P = 0.013)\) was found in the expression of \(\text{IGF2}\); however, Tukey's \textit{post hoc} comparison did not identify individual differences between means. The expression of \(\text{MYOD1}\) tended \((P = 0.056)\) to be lower in L- than M-pigs \((L = 1.96 \text{ vs. } M = 1.86)\), and a tendency \((P = 0.091)\) to a DIET × BtW interaction effect on \(\text{PRKAA2}\) expression was found, where M-piglets from Ctrl sows displayed the lowest expression compared with the remaining piglets \((\text{M-Ctrl} = 0.44; \text{L-Ctrl} = 0.53; \text{M-ARG} = 0.59; \text{L-ARG} = 0.60 \text{ relative expression level})\). Again, there were no differences in individual interaction means identified by the Tukey's \textit{post hoc} comparison.

### Discussion

The increased litter size of modern sow breeds is likely associated with increased incidents of IUC and consequently IUGR. Therefore, an increased proportion of underdeveloped piglets within the litters can be observed [21, 22]. These piglets display a lower postnatal survival rate [23], and a poorer growth performance compared with their larger littermates [24, 25]. These impairments can in part be attributed to the reduced myofiber hyperplasia mainly due to lower formation of S myofibers [8]. One approach to enhance hyperplasia is by supplementing L-arginine to the sows during early gestation, shown to be leading to increased formation of P myofibers, which serve as a scaffold for the development of the S myofibers [13]. To alter the intrauterine environment, either unilateral ovary-hysterectomy or unilateral oviduct ligation of sows can be applied, where sows subjected to the latter procedure have shown to display minimal crowding and adequate placental development.
Table 5 Myofiber characteristics in the Semitendinosus muscle of selected offspring born from oviduct ligated (OL; non crowded) and intact (IN; relatively crowded) sows fed an unsupplemented (Ctrl) or l-arginine supplemented diet (ARG) from d 14 to 28 of gestation

| Item                        | IN Ctrl | ARG | OL Ctrl | ARG | SEM | IUC | DIET | BtW | IUC×DIET | DIET×BtW |
|-----------------------------|---------|-----|---------|-----|-----|-----|------|-----|----------|----------|
| Observations                | 16      | 17  | 19      | 17  | 15  |     |      |     |          |          |
| Muscle area, μm² x 10⁻²     |         |     |         |     |     |     |      |     |          |          |
| Total                       | 5.762   | 6.780 | 7.505   | 8.795 | 0.9429 | 0.144 | 0.003 | 0.653 | 0.738   | 0.053    |
| Dark portion                | 2.359   | 2.278² | 2.772² | 3.572³ | 0.2931 | 0.078 | 0.025 | 0.233 | 0.007   | 0.573    |
| Light portion               | 3.151   | 4.118 | 4.906   | 5.677 | 0.8279 | 0.143 | 0.011 | 0.910 | 0.786   | 0.061    |
| Myofiber number, N          |         |     |         |     |     |     |      |     |          |          |
| Total muscle                | 476,025 | 527,711 | 536,451 | 607,928 | 35,511.0 | 0.236 | 0.003 | 0.136 | 0.604   | 0.475    |
| Dark portion                | 193,928 | 198,254 | 184,590 | 218,604 | 22,898.2 | 0.884 | 0.131 | 0.002 | 0.231   | 0.560    |
| Primary myofibers (P)       | 6,105   | 5,840 | 5,868   | 6,156 | 638.8 | 0.970 | 0.974 | 0.069 | 0.242   | 0.573    |
| Secondary myofibers (S)     | 187,823 | 192,414 | 178,722 | 212,448 | 22,455.9 | 0.883 | 0.124 | 0.002 | 0.231   | 0.559    |
| S:P ratio                   | 30.8    | 33.2 | 30.6    | 34.4 | 2.86  | 0.912 | 0.051 | 0.356 | 0.643   | 0.337    |

Table 6 Relative expression of myogenes related genes in the Semitendinosus muscle of selected offspring born from oviduct ligated (OL; non crowded) and intact (IN; relatively crowded) sows fed an unsupplemented (Ctrl) or l-arginine supplemented diet (ARG) from d 14 to 28 of gestation

| Gene⁶ | IN Ctrl | ARG | OL Ctrl | ARG | SEM | IUC | DIET | BtW | IUC×DIET | DIET×BtW |
|-------|---------|-----|---------|-----|-----|-----|------|-----|----------|----------|
| Observations | 16 | 17  | 19      | 17  | 15  |     |      |     |          |          |
| IGF2  | 2.36    | 1.55 | 0.91    | 1.70 | 0.555 | 0.487 | 0.967 | 0.965 | 0.013    | 0.950    |
| IGFBP5| 5.63    | 5.65 | 2.32    | 4.62 | 2.190 | 0.489 | 0.233 | 0.275 | 0.290    | 0.297    |
| MSTN⁴ | 1.60    | 1.64 | 0.46    | 0.98 | 1.754 | 0.351 | 0.232 | 0.105 | 0.241    | 0.110    |
| MYF5⁴ | 0.35    | 0.42 | 0.24    | 0.28 | 1.694 | 0.631 | 0.531 | 0.895 | 0.974    | 0.159    |
| MYF6  | 0.58    | 0.65 | 1.18    | 0.98 | 0.576 | 0.623 | 0.859 | 0.893 | 0.664    | 0.631    |
| MYOD1⁴| 2.36    | 1.84 | 0.68    | 0.48 | 2.143 | 0.223 | 0.340 | 0.056 | 0.885    | 0.418    |
| MYOG  | 0.94    | 0.70 | 1.32    | 1.11 | 0.255 | 0.333 | 0.168 | 0.392 | 0.915    | 0.417    |
| PRKAA2| 0.72³   | 1.04³ | 0.26³   | 0.15³ | 0.194 | 0.024 | 0.264 | 0.298 | 0.034    | 0.091    |

[18]. Thus, compared with intact natural crowded sows as used in the current study, OL sows with average prolificacy are suitable models for investigating the consequences of IUC and IUGR in newborn piglets. Hence, intact prolific sows with naturally crowded uterus and OL sows with a “non-crowded uterus” were used in the present study to mimic two degrees of IUC and to investigate whether the positive effect of early gestational supplementation of the sows with l-arginine on myofiber hyperplasia observed in 75-day-old fetuses [13] would continue until birth and would differ depending on the extent of IUC.
Sow performance and litter characteristics

Important causes of IUGR are impaired placental development, including suboptimal vascularization of the placenta, increased numbers of fetuses due to high proliferation and uneven distribution of energy and dietary nutrients among the fetuses [26]. As indicated by the number of total and born alive piglets in combination with the corresponding BtW, IUGR occurred to a greater extent in IN sows compared with OL sows. A sow with a litter size of ≥ 15 is categorized as being high prolific [24, 27]. This was the case with the IN sows. On the contrary, OL sows had a litter size below the average herd level (9.7 vs. 12.3 live born). The OL sows compared with the IN sows gave birth to a smaller number of stillborn piglets, which was expected from previous findings associating increased litter size with a greater number of stillborn piglets [27]. Litter characteristics of IN sows were in agreement with results from a previous study where offspring developing in a crowded compared with an uncrowded intra-uterine environment exhibited phenotypes associated with IUGR [18]. In the present study, performance of OL sows with respect to litter size and average BtW was comparable to that observed in low to average prolific sows [22].

Effects of maternal dietary L-arginine supplementation on offspring have been previously investigated, but both dietary level, onset of supplementation during gestation and impact on sow and offspring traits have varied greatly among different studies as reviewed by Wu et al. [28]. Among reported effects of L-arginine are increased numbers of total born, born alive and total litter BtW in multiparous sows [29]. However, the sows included in the two former studies had an average litter size of 13 to 14 piglets, thus are to be categorized as average rather than highly prolific with no expected major impact of IUC and IUGR. In the present study, dietary supplementation of L-arginine did not significantly affect the sow reproductive performance. Nevertheless, numerical improvements of particularly the number of piglets born alive as well as a greater BtW of offspring in IN-ARG compared with IN-Ctrl sows were observed. However, the reproduction data must be interpreted with caution due to the low number of sows used in this study.

Muscle and organ weights of offspring

The greater BtW and weight of STM and PM of offspring from OL sows compared with IN sows were foreseen as it has been reviewed extensively that there is a high correlation between large litter size and extent of crowding as well as greater within-litter variation of BtW and greater number of low BtW offspring [2, 30, 31]. Organ weights of offspring were within the normal range of newborn piglets [18, 32]. The tendency for an increased absolute STM weight in offspring from ARG sows suggests that, independent of IUC, L-arginine supplementation promotes muscle development of offspring, an observation that has to the best of the authors’ knowledge not been shown in previous studies.

Besides reduced BtW, brain:liver weight ratio has been shown to positively correlate with uterine crowding [11] and therefore is an indicator of IUGR [33]. The brain-sparing mechanism ensures steady relative brain weight development when maternal nutrient supply does not match requirements for adequate fetal development [34]. The greater liver weight combined with the similar brain weight resulted in a lower brain:liver weight ratio in offspring from ARG sows compared with Ctrl sows and supports the hypothesis that L-arginine supplementation has the potential to alleviate the negative impact of IUGR. In addition, the absolute heart weight was only numerically greater in ARG compared with Ctrl, an observation that has also been related to low birth weight pigs at slaughter age [35]. Thus, with the exception of the brain:liver ratio, this observation would not support the positive effect of L-arginine on organ development under crowding conditions.

Due to the rather high level and intake of crude protein and supplementation of daily L-arginine, a great load of maternal arginine could be expected. However, as discussed above, no detrimental effects on either the sow or the offspring traits were observed, indicating extensive catabolism of arginine and that maternal intake of arginine was not excessive [36]. Thus, it was concluded that the level of supplemented arginine was within the safety margin eliciting no toxicity or adverse effects [37, 38], whereas higher crude protein level and arginine concentration of the diet might lead to impaired reproductive performance, antagonism between amino acids and toxicity of ammonia to sows and their fetuses [28].

Myofiber-related traits of the Semitendinosus muscle

It is widely accepted that the formation of S myofibers is highly dependent on fetal nutrient uptake [12]. Thus, a reduced nutrient availability in the placenta during mid to late gestation, is likely to result in a lower S myofiber hyperplasia. Previous studies showed that a lower number of myofibers was associated with reduced muscle mass at weaning and at slaughter age [6, 39] meaning an ongoing disadvantage during the entire fattening performance. In the present study, supplementing L-arginine to the sows during gestation resulted in an increased TNF, with the greatest contribution derived from the second wave of myofiber formation as indicated by the greater S:P ratio in the STM4 and the greater TNF in the STM4. In comparison, a similar previous study reported that early gestational supplementation with L-arginine enhanced prenatal hyperplasia, but,
in contrast, the main increase of TNF originated from the formation of P myofibers [13]. However, in that study myofiber hyperplasia was assessed at d 75 of gestation, a time point where hyperplasia of S myofibers is still ongoing. It is worth mentioning that the increased hyperplasia observed in offspring from ARG sows was independent of BtW (L-ARG: 529,186 vs. M-ARG: 606,453; \( P = 0.136 \)). This finding contradicts our hypothesis that underprivileged fetuses would preferentially benefit from ARG supplementation by an increased placental vascularization. Results of a recent study [40] suggested that supplementing the gestational diet with either L-arginine, ractopamine, or a combination of the two increased myofiber diameter, indicating that supplementation had an effect on muscle fiber hypertrophy rather than hyperplasia. Ultimately, this resulted in a lower proportion of low BtW piglets (< 0.8–1.2 kg BtW), and an increased proportion of medium and high BtW piglets (> 1.2–1.6 kg BtW) from L-arginine supplemented compared with unsupplemented sows [40]. However, interestingly and in contrast to the present study, a lower TNF was observed in offspring from supplemented compared with unsupplemented sows. One explanation for the discrepancy between the observation in our study and the study of Garbossa et al. [40] could be the mode of L-arginine supplementation, either during d 14-28 or 25-53 of gestation, respectively, and at either 0.89% or 1.00% of the diet, respectively. With these combined results in mind, it is relevant to reflect on the extent to which supplementary L-arginine is directing dietary energy for hyperplasia or hypertrophy in fetuses during the different phases of gestation. Furthermore, investigating the impact of gestational supplementation on hyperplasia especially in post-weaning pigs would be of great importance, as results on this matter are contradictory. For instance, supplementing L-carnitine daily to L-BtW piglets during the suckling period resulted in a third wave of hyperplasia [41] during the nursing period, but no difference in TNF was found between control and L-carnitine supplemented pigs at the age of slaughter [42]. Based on this contradiction, one could speculate that changes occurring during the prenatal period are more manifested and less prone to environmental changes in the postnatal period, thus, having permanent positive effects until the animal reaches slaughter weight.

In light of this discussion and based on earlier findings [43, 44], one can assume that maternal arginine concentration was increased in plasma of sows fed L-arginine during the gestational period from d 14-28.

**Gene expression of myogenesis-related genes in the Semitendinosus muscle**

Muscle development during the perinatal period requires the regulation of several myogenesis related genes [45]. In the present study none of the genes analyzed were responding to the extent of IUC or dietary treatment, except for PRKAA2, the expression of which was up-regulated in the STM of IN offspring compared with OL offspring. PRKAA2 is a known inhibitor of muscle protein synthesis [46], and its greater expression in IN offspring is consistent with their lower BtW and relative STM weight emphasizing the importance of this gene in pre-natal muscle development. In contrast to the increased TNF at birth elicited by maternal L-arginine supplementation, results from the gene expression analysis did not support a myogenic effect at the molecular level. As hyperplasia ceases around d 90 of gestation, genes involved in myogenesis including myofiber formation might no longer show a specific up-regulation, but would rather be expressed at a steady state at birth when the STM was sampled. However, although there was no direct effect of L-arginine supplementation on the gene expression of myogenesis-related genes, it appears that IGF2 and PRKAA2 expressions in neonate STM could be influenced by the dietary level of this particular amino acid in certain uterine environments because there was a significant interaction.

**Conclusion**

In conclusion, the present study further adds to the increasing evidence that intra-uterine crowding prevalent in high prolific sows critically affects the phenotype of the offspring. This was manifested by the low BtW in contrast to the moderate litter size and offspring BtW of the OL counterparts. Confirming our first hypothesis, L-arginine in the early gestational diet seems to reduce the negative impacts of IUGR, as shown by the increased hyperplasia, body weight and STM area of the offspring at birth. As muscle area increased more than TNF, L-arginine supplementation obviously not only enhanced prenatal myofiber hyperplasia but also hypertrophy. However, details concerning the way in which L-arginine affects myofiber development on cellular and molecular levels remain to be determined. The second hypothesis that L-arginine would be especially efficient in L-BtW piglets was not confirmed. Still, this feeding strategy could be of great benefit to especially L-BtW pigs as they are particularly vulnerable. Thus, L-arginine supplementation to sows in early gestation would potentially improve the survival rate during nursing, and the growth potential during fattening of L-BtW piglets.

**Abbreviations**

ARG: Arginine supplementation; BtW: Birth weight; Ctrl: Alanine supplementation; DIET: Dietary treatment; IGF2: Insulin-like growth factor 2; IGFBP5: Insulin-like growth factor binding protein 5; IN: Intact sows; IUC: Intra-uterine crowding; IUGR: Intra-uterine growth retardation; L: Low; M: Intermediate; MSTN: Myostatin; MYF5: Myogenic factor 5; MYF6: Myogenic factor 6; MYOD1: Myogenic differentiation 1; MYOG: Myogenin (myogenic factor 4); OL: Unilateral oviduct ligated; P: Primary; PM: Psoas major;
References

1. Tschircher M, Puppe B, Tschircher A, Tiemann U. Early identification of neonates at risk: Trials of newborn pigs with respect to survival. Theriogenology. 2000;54(3):371–88. doi:10.1016/S0093-691X(00)00355-1.

2. Foxcroft GR, Bee G, Dixon WT, Hahn M, Harding J, Patterson J, et al. Consequences of selection for litter size on piglet development. In: Wiseman J, Varley MA, McClintock S, Kemp B, editors. Paradigms in Pig Science. Nottingham: Nottingham Univ. Press; 2007. p. 207–29.

3. Rekiel A, Wloczek J, Batosinska M, Kulisiewicz J. Effect of sow prolificacy and nutrition on preand postnatal growth of progeny – a review. Anim. Anim. Sci. 2014;14(1):1–15. doi:10.2478/aas-2013-0060.

4. Bérard J, Pardo CE, Béatrice S, Kreuzer M, Bee G. Intrauterine crowding decreases average birth weight and affects muscle fiber hyperplasia in pigs. J Anim Sci. 2010;88:3242–50. doi:10.2527/jas.2010-2867.

5. Dwyer CM, Fletch JM, Stickland NC. Muscle cellularity and postnatal growth and carcass quality in pigs as related to myogenesis. J Anim Sci. 2006;84(Suppl E13–23). doi:10.2527/2006.84E13_supplE13x.

6. Swatland HJ. Muscle growth in the fetal and neonatal pig. J Anim Sci. 1973;37(2):536–45. doi:10.2527/jas1973.376536x.

7. Ashmore CR, Addis PB, Doerr L. Development of muscle fibers in the fetal pig. J Anim Sci. 1973;36(6):1086–93. doi:10.2527/jas1973.3661088x.

8. Beermann DH, Cassens RG, Hausman GJ. A second look at fiber type differentiation in porcine skeletal muscle. J Anim Sci. 1978;46(1):125–32. doi:10.2527/jas1978.461125x.

9. Town SC, Putman CT, Tuchinsky NJ, Dixon WT, Foxcroft GR. Number of conceptuses in utero affects porcine fetal muscle development. Reproduction. 2004;128(4):443–54. doi:10.1530/rep.1.00069.

10. Wigmore PM, Stickland NC. Muscle development in large and small pig fetuses. J Anat. 1983;137(Pt 2):235–45.

11. Beermann DH, Hausman GJ, McKinnon KN, Liu YP, Chen HD, Chen J. Effects of dietary L-arginine supplementation on growth performance, carcass quality, and eating quality of pork. J Anim Sci. 2000;78(2):475–80. doi:10.2527/2000-0202.

12. Foxcroft GR, Town SC. Prenatal programming of postnatal development in the pig. Soc Reprod Fertil Suppl. 2006;65(3):261–70. doi:10.2531/jsp00726-06520000151-2.

13. Bérard J, Reid DA. Utero-placental vascular development and placental function. J Anim Sci. 2000;78(6):1689–91. doi:10.2527/2000-0608.

14. Wiener R, silacci P, Cianci C, Silacci D, Alabadi A. Impact of dietary L-arginine supplementation on placental growth in continuous sows. J Reprod Dev. 2010;56:1–15. doi:10.1530/rep.1.00069.

15. Wu GY, Bazer FW, Johnson GA, Burghardt RC, Erikson DW, Frank JW, et al. Dietary supplementation with 0.8% L-arginine between days 0 and 25 of gestation reduces litter size in pigs. J Nutr. 2010;140(6):1111–6. doi:10.3945/jn.110.121350.

16. Reynolds LP, Redmer DA. Uterine-placental vascular development and placental function. J Anim Sci. 1975;40(6):1659–91. doi:10.2527/1975.4061659.

17. Hazeleger W, Ramakers RM, Snits C, Kemp B. Effect of Progenos on placenta and fetal development in pigs. J Anim Sci. 2007;85(8).

18. Pardo CE, Bérard J, Kreuzer M, Bee G. Intrauterine crowding in pigs impairs formation and growth of secondary myofibers. Anim. 2013;73(3):430–8. doi:10.1159/00037110001802.

19. Ashmore CR, Addis PB, Doerr L. Development of muscle fibers in the fetal pig. J Anim Sci. 1973;36:1086–93. doi:10.2527/jas1973.3661088x.

20. Beermann DH, Cassens RG, Hausman GJ. A second look at fiber type differentiation in porcine skeletal muscle. J Anim Sci. 1978;46(1):125–32. doi:10.2527/jas1978.461125x.

21. Town SC, Putman CT, Tuchinsky NJ, Dixon WT, Foxcroft GR. Number of conceptuses in utero affects porcine fetal muscle development. Reproduction. 2004;128(4):443–54. doi:10.1530/rep.1.00069.

22. Foxcroft GR, Town SC. Prenatal programming of postnatal development - the unseen cause of variance. Advances in Pork Production. 2004;15:269.

23. Gao K, Jiang Z, Lin Y, Zheng C, Zhou G, Chen F, et al. Dietary L-arginine supplementation enhances placental growth and reproductive performance in sows. Amino Acids. 2012;44(6):2207–14. doi:10.1007/s00726-011-1200-9.

24. Foxcroft GR, Dixon WT, Dyck MK, Novak S, Harding JC, Almeida FC. Prenatal programming of postnatal development in the pig. Soc Reprod Fertil Suppl. 2009;66:213–31.

25. Wu GY, Bazer FW, Satterfield MC, Li XL, Wang XQ, Johnson GA, et al. Impacts of arginine nutrition on embryonic and fetal development in mammals. Amino Acids. 2013;45(2):241–56. doi:10.1007/s00726-013-1515-2.

26. Wu GY, Bazer FW, Wallace JM, Spencer TE. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. J Anim Sci. 2007;85(9):2409–18. doi:10.2527/jas.2007-0576.

27. Foxcroft GR, Town SC. Prenatal programming of postnatal performance - the unseen cause of variance. Advances in Pork Production. 2004;15:269.

28. Wu GY, Bazer FW, Wallace JM, Spencer TE. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. J Anim Sci. 2000;78(2):475–80. doi:10.2527/2000-0202.

29. Wu GY, Bazer FW, Johnson GA, Burghardt RC, Erikson DW, Frank JW, et al. Dietary supplementation with 0.8% L-arginine between days 0 and 25 of gestation reduces litter size in pigs. J Nutr. 2010;140(6):1111–6. doi:10.3945/jn.110.121350.

30. Wu GY, Morris SM. Arginine metabolism: nitric oxide and beyond. Biochem. J. 1998;336:1–17. doi:10.1042/p3360001.

31. Beermann DH, Hausman GJ, McKinnon KN, Liu YP, Chen HD, Chen J. Effects of dietary L-arginine supplementation on growth performance, carcass quality, and eating quality of pork. J Anim Sci. 2000;78(6):1689–91. doi:10.2527/2000-0608.

32. Beermann DH, Hausman GJ, McKinnon KN, Liu YP, Chen HD, Chen J. Effects of dietary L-arginine supplementation on growth performance, carcass quality, and eating quality of pork. J Anim Sci. 2000;78(6):1689–91. doi:10.2527/2000-0608.
33. Town SC, Patterson JL, Pereira CZ, Gouley G, Foxcroft GR. Embryonic and fetal development in a commercial dam-line genotype. Anim. Reprod. Sci. 2005;85(3-4):301–16. doi:10.1016/j.anireprosci.2004.05.019.

34. DeLong GR. Effects of nutrition on brain development in humans. Am J Clin Nutr. 1993;57(2):2865–905.

35. Rehfeldt C, Tuchscherer A, Hartung M, Kuhn G. A second look at the influence of birth weight on carcass and meat quality in pigs. Meat Sci. 2008;78(3):170–5. doi:10.1016/j.meatsci.2007.05.029.

36. Hou Y, Yao K, Yin Y, Wu G. Endogenous Synthesis of Amino Acids Limits Growth, Lactation, and Reproduction in Animals. Advances in Nutrition: An International Review Journal. 2016;7(2):331–42. doi:10.3845/an.115.010850.

37. Wu G, Bazer FW, Cudd TA, Jobgen WS, Kim SW, Lassala A, et al. Pharmacokinetics and safety of arginine supplementation in animals. J Nutr. 2007;137(6):1673–1680.

38. Wu Z, Hou Y, Hu S, Bazer FW, Meeniger C, McNeal CJ, et al. Catabolism and safety of supplemental L-arginine in animals. Amino acids. 2016;1–12. doi:10.1007/s00726-016-2245-9.

39. Rehfeldt C, Lang IS, Gori S, Hennig U, Kelbe C, Stabenow B, et al. Limited and excess dietary protein during gestation affects growth and compositional traits in gilts and impairs offspring fetal growth. J Anim Sci. 2011;89(2):329–41. doi:10.2527/jas.2010-2970.

40. Garbossa CA, Junior FM, Silveira H, Faria PB, Schinkel AP, Abreu ML, et al. Effects of ractopamine and arginine dietary supplementation for sows on growth performance and carcass quality of their progenies. J Anim Sci. 2015;93(6):2872–84. doi:10.2527/jas.2014-8824.

41. Løyd D, Kelbe C, Rehfeldt C. L-Carnitine supplementation during suckling intensifies the early postnatal skeletal myofiber formation in piglets of low birth weight. J Anim Sci. 2009;87(7):2216–26. doi:10.2527/jas.2008-1662.

42. Løyd D, Rehfeldt C. Effects of L-carnitine supplementation to suckling piglets on carcass and meat quality at market age. Animal. 2013;7(7):1191–8. doi:10.1017/S1751731113000268.

43. Mateo RD, Wu G, Bazer FW, Park J, Shinzato I, Kim SW. Dietary L-arginine supplementation enhances the reproductive performance of gilts. J Nutr. 2007;137(3):652–6.

44. Li X, Bazer FW, Johnson GA, Burghardt RC, Frank JW, Dai Z, et al. Dietary supplementation with L-arginine between days 14 and 25 of gestation enhances embryonic development and survival in gilts. Amino Acids. 2014;46(2):375–84. doi:10.1007/s00726-013-1626-6.

45. Pas MFW, Wit AAW, Priem J, Cagnazzo M, Davoli R, Russo V, et al. Transcriptome Expression Profiles in Prenatal Pigs in Relation to Myogenesis. J Muscle Res Cell Motil. 2005;26(2):157–65. doi:10.1007/s10974-005-7004-6.

46. Bolster DR, Crozier SJ, Kimball SR, Jefferson LS. AMP-activated Protein Kinase Suppresses Protein Synthesis in Rat Skeletal Muscle through Down-regulated Mammalian Target of Rapamycin (mTOR) Signaling. J Biol Chem. 2002;277(21):19977–80. doi:10.1074/jbc.C200171200.

47. Foxcroft GR, Dixon WT, Novak S, Putman CT, Town SC, Vinsky MDA. The biological basis for prenatal programming of postnatal performance in pigs. J Anim Sci. 2006;84(E. Suppl):E105–12. doi:10.2527/2006.8413_suppe105x.