Influence of L-carnitine and L-arginine on protein synthesis and maturation of the semitendinosus muscle of lightweight piglets

J. G. Madsen¹,² | E. Seoni¹ | M. Kreuzer² | P. Silacci¹ | G. Bee¹

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

Summary
Lightweight (LW) piglets from large litters display impaired growth performance compared with heavier littermates. This study investigated the growth performance and muscle development of early-weaned LW piglets (birthweight < 1.2 kg) from large litters (17.3 ± 3.0 total born per litter), fed ad libitum a milk replacer supplemented with either L-carnitine (CAR) or L-arginine (ARG) from day 7 to day 28 of age. In total, 36 female and entire male Swiss Large White piglets, weaned on day 7 of age, were artificially reared in pairs in rescue decks. They were allocated to one of three dietary treatments: unsupplemented control (CON), 0.48 g L-carnitine·piglet⁻¹·day⁻¹ (CAR) or 1.20 g L-arginine·kg⁻¹·day⁻¹ (ARG). Milk replacer was prepared daily in a 1:4 powder-to-water ratio and fed ad libitum. Piglets were weighed at birth and on days 7, 14, 21 and 28. Feed intake was assessed daily. Piglets were euthanized on day 28. The entire semitendinosus muscle (STM) was collected, and organs were weighed. Subsequently, the STM was divided into the light (STMₗ) and dark (STMₐ) portion, and contractile and metabolic traits were analysed by ATP histochemistry, enzyme activities and gene expression. No differences in growth performance, organ and STM weight and on contractile traits were found between groups. A tendency (p < .10) for an elevated lipid oxidation enzyme activity in the STMₗ and STMₐ and greater (p < .05) phosphorylation of the mammalian target of rapamycin pathway in the STMₗ of CAR compared with CON piglets was found. Despite these metabolic responses, the lack of effect of CAR and ARG supplementation on growth performance suggests that providing the milk replacer ad libitum in combination with added CAR and ARG is insufficient for eliciting faster growth of LW piglets.

KEYWORDS
amino acid, artificial rearing, growth performance, litter size, milk replacer

1 | INTRODUCTION

Increased litter size, a large within-litter variation and decrease in the average birthweight (BtW) of the litter are all consequences of selection for hyperprolific sows in the commercial pig production (Rutherford et al., 2013). Simultaneously in such sows, milk yield and number of functioning teats does no longer correspond to the number of piglets in the litter (Andersen, Naevdal, & Boe, 2011). This compromises the growth of the piglets in a way that they are unable to reach their full potential (Harrell, Thomas, & Boyd, 1993). Furthermore, lightweight (LW) piglets display impaired growth performance in the nursery (Deen & Bilkei, 2004) and in the post-weaning growth phase (Madsen & Bee, 2015). In addition, LW piglets have been shown to exhibit a lower number of myofibres compared with their heavier littermates (Bérard, Pardo, Bethaz, Kreuzer, & Bee, 2010), partly contributing to their reduced post-natal growth potential. The LW piglets also exhibit a lower survival rate...
compared with the litter average (Quiniou, Dagorn, & Gaudré, 2002), where the main cause of death across all BtW categories is documented to be crushing by the sow (Edwards, 2002). However, it is argued that due to undernourishment, LW piglets reside for longer periods in close proximity of the sow, thereby increasing the risk of crushing (Weary, Pajor, Thompson, & Fraser, 1996). In this context, early artificial rearing by relocating excess piglets to rescue decks and feeding them on milk replacer will naturally remove their risk of being crushed by the sow. Applying milk replacer to early-weaned piglets has been shown to improve daily gain of medium and heavy birthweight piglets (Zijlstra, Whang, Easter, & Odle, 1996), suggesting it to be a viable alternative to insufficient sow milk yield. However, one important disadvantage of available commercial milk replacers is the suboptimal nutritional and amino acid composition compared with the sows’ milk (Mavromichalis, Parr, Gabert, & Baker, 2001; Theil, Kristensen, Jorgensen, Labouriau, & Jakobsen, 2007). Being important for growth (Kim & Wu, 2004), the semi-essential amino acid arginine (ARG) is involved in the protein synthesis signalling pathway of the mammalian target of rapamycin (mTOR) (Yao et al., 2008). Its content is substantially lower in commercial milk replacer compared with sow milk (0.7 vs. 1.3 g/kg DM). L-carnitine (CAR) is another compound which when fed to conventionally reared piglets, has shown to increase the number of myofibres in the early postnatal period (Lösel, Kalbe, & Rehfeldt, 2009). In a recent study, we observed a greater ratio of lactate dehydrogenase to either citrate synthase with CAR and β-hydroxyacyl-CoA dehydrogenase activity with ARG-supplemented milk replacers (Madsen, Kreuzer, & Bee, 2014). Thus, the relative importance of the glycolytic compared with the oxidative pathway was greater in the semitendinosus muscle of supplemented LW pigs suggesting that these supplements were beneficial for muscle maturation. However, because in that study milk replacer was offered restrictively several times a day based on previous recommendations (60 g DM/kg body weight (BW) (Kim & Wu, 2004)), weaning weights at day 28 were low and independent of the dietary treatment. In the present study, the hypothesis was to test the effect of L-arginine and L-carnitine supplements to milk replacer under ad libitum feeding conditions in combination with artificial rearing during the nursery phase on both muscle development and growth performance of LW piglets from hyperprolific sows.

2 | MATERIALS AND METHODS

All procedures involving animals used in this study was approved by The Cantonal Committee for Animal Care and Use (2012_39_FR). Every day prior to feeding all piglets were controlled for health condition. In case of diarrhoea, they were treated with either Borgal (Virbac group, Carros, France) or Colivet (Prodivet pharmaceuticals, Hagbenden, Belgium). In rare severe cases, both Borgal and Colivet was provided.

2.1 | Animals, treatments and experimental conditions

A total of 36 piglets (18 females and 18 entire males) from Swiss Large White sows (parity one to seven) were used in the experiment. Piglets originated from 13 litters with an average size of 17.3 ± 3.0 (mean ± standard deviation) total born piglets and average litter BW of 1.27 ± 0.10 kg. Piglets with the lowest day 7 body weight (BW), on average weighing 1.18 ± 0.15 kg, were selected. These were often, but not exclusively, those with the lowest BtW. Always two piglets were weaned together on day 7 after birth. Selection aimed at achieving similar BW and day-7 BW within couple of piglets and dietary treatments (realized: control (CON): 2.11 ± 0.17 kg, CAR: 2.03 ± 0.35 kg and ARG: 2.12 ± 0.34). Piglets were weaned in customized rescue decks (0.49 m × 1.1 m, semi-slatted floor), and remained there for 21 days. The customized rescue decks were designed to fulfil legal space requirements. The selected piglets were blocked by BW and day-7 BW and randomly allocated within block to one of the three dietary treatments: CON (n = 12), CAR (n = 12) and ARG (n = 12). Sex was balanced between blocks with six males and six females in each dietary treatment. To acquire the desired number of animals, three farrowing series were needed.

2.2 | Diet and feeding regime

The main ingredients of the milk replacer consisted of cow milk products (Table 1). The milk replacer of the ARG treatment was supplemented with 1.20 g L-arginine HCI·kg BW\(^{-1}\)·day\(^{-1}\) (Evonik Degussa GmbH, Hanau, Germany) based on requirements for suckling piglets given by Wu, Meininger, Knabe, Bazer, and Rhoads (2000). In contrast, L-carnitine (0.48 g·piglet\(^{-1}\)·day\(^{-1}\)) (Carniking, Lohmann Animal Health, Cuxhaven, Germany) was offered to the piglets of the CAR group each morning at 08.00 h by putting it into the feeding cup (Provimi BV, Rotterdam, the Netherlands). To make the CON and CAR diets isonitrogenous an appropriate amount of L-alanine (Evonik Degussa GmbH, Hanau, Germany) was added. The milk replacer was prepared fresh every morning in a 1:4 powder-to-water (lukewarm) ratio and offered ad libitum. In addition, as a preventive measure of diarrhoea, 4 ml of Globigen (EW Nutrition GmbH, Visbek, Germany) containing egg powder-derived immunoglobulins was put into the feeding cup each morning. Piglets were weighed at birth and on days 7, 14, 21 and 28 of age.

The calculated weaning weight of conventionally reared LW piglets (BW range: 0.810–1.190 kg) from the Agroscope research herd was based on recordings obtained from December 2004 to November 2016 of 4318 piglets originating from sows with >15 total born piglets per litter.

2.3 | Muscle tissue and organ sampling

On day 28 of age, the piglets were anaesthetized with an isoflurane-oxygen mixture [4% vol/vol] and euthanized by exsanguination. After exsanguination, piglets were dissected to collect and weigh various organs. The whole semitendinosus muscle (STM) from the right carcass side was excised. The STM was weighed and length and circumference at the midpoint of the muscle was measured. Circumference was used to calculate the area of the entire muscle area. The STM was then divided into the dark (STM\(_d\)) and light (STM\(_l\)) portion. From each portion a
TABLE 1 | Ingredient and analysed nutrient composition of basic milk replacer and sow milk

| Ingredient          | Basic milk replacer (n = 20) | Sow milk (n = 36) |
|---------------------|-----------------------------|------------------|
| Ingredients, g/kg as fed |                            |                  |
| Cow whey powder     | 624                         | –                |
| Whole cow milk powder | 288                         | –                |
| Cow milk protein    | 62                          | –                |
| Dextrose (feed grade) | 10                          | –                |
| Dicalcium phosphate | 10                          | –                |
| L-methionine        | 1                           | –                |
| l-lysine HCl        | 1                           | –                |
| Vitamin–mineral premixa | 4                           | –                |
| Analysed chemical composition, g/kg dry matter (DM) |         |                  |
| DM, g/kg wet weight | 206.7 ± 0.1                  | 210.9 ± 18.4     |
| Total ash           | 81.7 ± 3.5                   | 43.8 ± 2.8       |
| Ether extract       | 76.9 ± 3.3                   | 420.9 ± 38.6     |
| Crude protein       | 201.3 ± 3.7                  | 279.5 ± 12.1     |
| Gross energy, MJ/kg DM | 18.1 ± 0.1                  | 27.6 ± 0.9       |
| Amino acids         |                             |                  |
| Alanine             | 7.7 ± 0.2                    | 9.4 ± 0.5        |
| Arginine            | 6.4 ± 0.2                    | 13.0 ± 0.7       |
| Aspartic acid       | 17.1 ± 0.4                   | 21.2 ± 1.2       |
| Cysteine            | 2.6 ± 0.1                    | 3.1 ± 0.2        |
| Glutamic acid       | 38.3 ± 1.0                   | 49.1 ± 2.5       |
| Glycine             | 4.2 ± 0.2                    | 8.6 ± 0.5        |
| Histidine           | 4.8 ± 0.2                    | 7.4 ± 0.4        |
| Isoleucine          | 11.2 ± 0.3                   | 11.4 ± 0.6       |
| Leucine             | 19.3 ± 0.5                   | 22.3 ± 1.2       |
| Lysine              | 17.1 ± 0.7                   | 20.3 ± 1.0       |
| Methionine          | 5.2 ± 0.2                    | 5.4 ± 0.2        |
| Phenylalanine       | 8.6 ± 0.2                    | 11.2 ± 0.5       |
| Proline             | 16.7 ± 0.5                   | 29.9 ± 1.4       |
| Serine              | 10.2 ± 0.3                   | 13.3 ± 0.7       |
| Threonine           | 10.4 ± 0.3                   | 11.2 ± 0.6       |
| Tryptophan          | 3.0 ± 0.1                    | 3.2 ± 0.2        |
| Tyrosine            | 7.8 ± 0.3                    | 11.5 ± 0.6       |
| Valine              | 12.3 ± 0.3                   | 14.5 ± 0.8       |
| Ratio in relation to lysine, g/g |             |                  |
| Arginine            | 0.37 ± 0.20                  | 0.64 ± 0.78      |
| Histidine           | 0.28 ± 0.20                  | 0.36 ± 0.38      |
| Isoleucine          | 0.66 ± 0.46                  | 0.56 ± 0.61      |
| Leucine             | 1.13 ± 0.70                  | 1.10 ± 1.29      |

*In the arginine and carnitine group, milk replacer was supplemented with l-arginine to a final concentration of 1.20 g l-arginine·kg BW−1·day−1 and with carnitine at 0.48 g l-carnitine·piglet−1·day−1, respectively.

Supplied per kg of diet: 20 mg Cu; 100 mg Fe; 50 mg Mn; 275 mg Zn; 0.75 mg I; 0.75 mg Se; 20,000 IU vitamin A; 2,000 IU vitamin D3; 100 mg choline; 10 mg vitamin B1; 15 mg vitamin B2; 75 mg vitamin B3; 75 mg vitamin B5; 15 mg vitamin B6; 0.005 mg vitamin B12; 2.5 mg vitamin Bp3; 0.1 mg vitamin B12; 325 mg vitamin E; 5 mg vitamin K3.

was extracted. After removal of organs and STM, the left carcass side was weighed and cooled at 3°C. The cooled left carcass sides, complete with skin and bones, were cut into smaller pieces by a knife and homogenized in a primary step with a meat mincer (R-2 version “A,” Robot-Coupe SNC, Montceau en Bourgogne Cedex, France). Minced carcass samples were then freeze-dried (Delta 1-24 LSC, Christ, Osterode am Harz, Germany) and cooled with liquid nitrogen for further homogenization with a Grindomix GM 200 (Retsch, Haan, Germany) prior to chemical analysis.

During the experiment about 50 ml of sow milk was collected from 36 hyperprolific sows (litter size >15 total born) of the Agroscope sow herd on day 21 after farrowing. Prior to sampling piglets were removed from the sow for 2 h. To initiate lactation the sows were injected into the neck with 1 ml of oxytocin (Intertocine-S, MSD Animal Health GmbH, Luzern, Switzerland).

### 2.4 | Analysis of sow milk, milk replacer and carcasses

Dry matter contents were determined by drying samples at 105°C for 160 min. Samples were subsequently analysed for total ash content by a thermogravimetric analyzer (Leco TGA-601, Leco Corporation, MI, USA). To determine the gross energy content of the samples, a bomb calorimeter (AC600 Semi-Automatic Calorimeter, Leco Corporation, MI, USA) was used. The nitrogen content of the milk replacer and sow milk was analysed according to the Dumas method (AOAC, 2012) using the automated CNS elemental analyzer (TruMac Series, Leco Corporation, MI, USA). In the carcasses, the nitrogen content was determined by the Kjeldahl (Kjeltec 2400/2460) method (AOAC, 2012), and crude protein content was calculated as 6.25 × nitrogen content. To determine ether extract in sow milk, milk replacer and carcasses, samples were digested by acid hydrolysis (10% HCl solution; Hydrotherm HT6, C. Gerhard, Königswinter, Germany) followed by a petroleum ether extraction (Speed Extractor 916, Büchi Labortechnik AG, Flawil, Switzerland) (ISO, 1999; VDLUFA, 1988). The amino acid composition of the milk replacer and sow milk was analysed by HPLC using the 2695 Alliance Separation Module coupled to the Alliance
Column Heater and the 2475 Multi-Lambda Fluorescence Detector (Waters Corporation, MA, USA) according to manual description (Waters AccQ Tag Chemistry Package 052874 TP, rev. 1).

2.5 | Histochemical analysis of the semitendinosus muscle

Myofibre characteristics of the STM were assessed as previously described by Bérard and Bee (2010). In brief, after equilibration for 30 min at −20°C, 10-μm-thick cross sections of the STMd and STMl were cut perpendicular to the fibre direction at −20°C in a cryotome (Shandon cryotome, Shandon Inc., PA, USA). Two sections per sample were mounted on glass microscopic slides (Menzel-Gläser Superfrost Plus, Gerhard Menzel GmbH, Braunschweig, Germany), air-dried for 60 min and stored at −20°C until use. Sections were then stained for the determination of myofibrillar ATPase activity after acid pre-incubation at pH 4.37. In both portions of the STM, type I myofibres stained dark and type Ila and type IIb myofibres stained grey and light respectively. Subsequently, stained sections were observed at 20 × magnification using a BX50 microscope in transmitted light mode (Olympus Optical Co., Hamburg, Germany) equipped with a high-resolution digital camera (ColorView12, Soft Imaging System GmbH, Münster, Germany) and captured as TIFF images. Average area and numbers of type I, type Ila and type IIb myofibres in the STMd and STMl were determined with the ANALYSIS software 3.0 image (Soft Imaging System GmbH, Münster, Germany). A minimum of 300 myofibres were counted per section. In addition to the average myofibre area, the relative area and relative number distribution, expressed as the percentage of the total area or total number of counted type I, Ila or IIb myofibres relative to the total area or total number of all myofibres counted within the STMd and STMl, was calculated. The total number of myofibres (TNF) was estimated by dividing the total number of type I, type IIIla and type IIIb myofibres in a defined area (0.14 mm²) of the STMd and STMl and extrapolating these numbers to the total muscle area.

2.6 | Enzyme activity analysis in the semitendinosus muscle

To characterize the metabolic properties of the STMd and STMl, the activity of citrate synthase (CS; EC 4.1.3.7), β-hydroxyacyl-CoA dehydrogenase (HAD; EC 1.1.1.35) and lactate dehydrogenase (LDH; EC 1.1.1.27) was measured. The CS, HAD and LDH represent key enzymes of the citric acid cycle, lipid oxidation and glycolysis respectively. Prior to activity assessment, protein was extracted from 50 to 100 μg of STMd and STMl samples stored at −80°C by lysing them in 500 μl of CellLytic MT buffer (Sigma-Aldrich Chemie GmbH, Buchs SG, Switzerland) supplemented with Complete TM Inhibitor Cocktail Tablets (F. Hoffmann-La Roche Ltd., Rotkreuz, Switzerland) and homogenized using the Precellys Lysing Kit CK 14 (Bertin Technologies, Montigny le Bretonneux, France) and Minilys Tissue Homogenizer (Bertin Technologies, Montigny le Bretonneux, France) at maximum speed for 40 s. Afterwards, the lysed tissue was transferred to a new tube and centrifuged at 12,000 g for 10 min at 4°C. The supernatant was stored at −80°C until further analysis. Protein concentration of the supernatant was determined with the Pierce Coomassie Plus (Bradford) Assay Kit (Thermo Scientific, DE, USA) using a microplate reader (Biochrom Asys UVM340, Biochrom Ltd., Cambridge, UK) equipped with the microWin 2000 software (HideX, Turku, Finland). Activities of LDH and CS were determined by applying the LDH and CS BioVision Activity Colorimetric Assay Kits (BioVision Incorporated, CA, USA) according to manufacturers’ protocols. Activities of LDH and CS were measured at 450 nm for 10 min and at 412 nm for 30 min, respectively, with a microplate reader (Biochrom Asys UVM340, Biochrom Ltd., Cambridge, UK) combined with the microWin 2000 software (HideX, Turku, Finland). The HAD activity was determined in a solution containing 97 mM/L potassium phosphate, 0.09 mM/L-β-acetoacetate-coenzyme A and 0.1 mM/L-β-nicotinamide adenine dinucleotide. The three reagents were mixed and the solution was incubated for 3 min at 37°C. The activity was measured at 340 nm with a spectrophotometer (Biochrom WPA Blowave II, Biochrom Ltd., Cambridge, UK) at initiation (0 min) and 3 min after the incubation. The final activity was based on the difference between the two time points. Samples were standardized per milligram protein and activity expressed as micromolar substrate degradation per minute.

2.7 | Determination of the phosphorylation of the mammalian target of rapamycin signalling pathway

To investigate activity of the mTOR signalling pathway, phosphorylation of residue Ser2481 was analysed in the STMd and STMl. First, for the immunoblotting approx. 100 μg of tissue from frozen STM, and STMd and STMl samples, respectively, was transferred to individual CK 14 tubes (Bertin Technologies, Montigny le Bretonneux, France) containing 400 μl of Cell Lytic MT Cell Lysis Reagent (Sigma-Aldrich Chemie GmbH, Buchs SG, Switzerland) supplemented with a protease–phosphatase inhibitor cocktail (Cell Signal Technology, MA, USA). Homogenization was afterwards performed by bead beating using the Minilys device (Bertin Technologies, Montigny le Bretonneux, France) with maximal agitation for 1 min. The homogenate was then centrifuged at 10,000 g for 10 min at 4°C. Subsequently, 100 μg of protein in the supernatant was subjected to electrophoresis on a 7% polyacrylamide gel, and later transferred to a polyvinylidene difluoride membrane (Western Bright PVDF L-08004-010, Witec, Luzern, Switzerland). In the step to detect the phosphorylated amount of mTOR, the gel was incubated for 12 h at 4°C with a rabbit polyclonal antiphospho mTOR primary antibody (Ser2481) (Cell Signal Technology, MA, USA) diluted 1:100 in phosphate-buffered saline supplemented with 0.1% Tween-20 (PBS-T). Membranes were then washed with PBS-T and further incubated for 1 h at room temperature with a goat anti-rabbit IgG HRP-conjugated secondary antibody (Sigma-Aldrich Chemie GmbH, Buchs SG, Switzerland) diluted 1:1000 in PBS-T. Luminescence was then developed using Western Blot Quantum kit (Witec, Luzern, Switzerland) following the manufacturer’s instructions, where visualization and quantification was performed in the GBox device using thegenesys software (Syngene, Cambridge, UK). After signal detection the membrane was immediately stripped with Restore Western Blot Stripping Buffer (Thermo Scientific, DE, USA) following the manufacturer’s instructions. In the step to detect
the total amount of mTOR, the membrane was subjected to a second round of hybridization where the primary antibody was a rabbit polyclonal anti-mTOR (Cell Signal Technology, MA, USA) diluted 1:100 in PBS-T. Results were expressed as ratios of the signal obtained with antiphosphor mTOR and total mTOR antibody respectively. To reduce variability between gels a reference sample was loaded on each gel, and ratios were normalized to the internal gel reference.

2.8 | Gene expression analysis in the semitendinosus muscle

Frozen tissue of STMd and STMl was homogenized in Precellys CK28 tubes (Bertin Instruments, Montigny le Bretonneux, France) containing QIAzol Lysis reagent (Qiagen, Hilden, Germany) using Minilys (Bertin Instruments, Montigny le Bretonneux, France) for 2 min at maximal speed. Total mRNA was further extracted using RNaseasy Plus Universal Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, and reverse-transcribed with QuantiTect Reverse Transcription kit. Primers for amplification of one protein synthesis-related gene (RPS6KB1) involved in the mTOR signalling pathway, and the myosin heavy chain (MyHC) isoform genes were designed using Primer blast (Ye et al., 2012). All primers were synthesized by Microsynth (Microsynth AG, Balgach, Switzerland), and primer accession numbers and sequences are listed in Table S1. Real-time PCR amplification was conducted using an Eco Illumina (Illumina, CA, USA) and eco study 5.0 software and expressed as relative expression over a reference sample.

2.9 | Statistical analysis

All data were tested for normality of residuals using the Univariate procedure of sas (Version 9.2, SAS Inst. Inc., NC, USA). As BtW and BW at day 7 did not differ between groups, they were not included as covariables for the growth performance data. Data on the weekly determined BW (days 7, 14, 21 and 28) and average daily gains (ADG) (weeks 2, 3 and 4 corresponding to the periods days 7–14, 14–21 and 21–28 respectively) were analysed with the MIXED procedure, applying the repeated statement and the first-order autoregressive covariance structure. Dietary treatment (CON, CAR, ARG), sex (entire

| TABLE 2 | Growth performance, feed intake and feed efficiency of early-weaned piglets born from hyperprolific sows with ad libitum access to an unsupplemented milk replacer or the same milk replacer supplemented with either \( l \)-carnitine or \( l \)-arginine from day 7 to day 28 of age 

| Item | Dietary treatment & Sex | p-value |
|-----|---------------------|---------|
|     | CON CAR ARG Male Female SEM | Trt S T Trt × T |
| Number | 12 12 12 18 18 |        |
| Body weight, kg |        |
| Day 7 | 2.03 2.06 2.13 2.11 2.04 | 0.100 |
| Day 14 | 3.12 2.80 3.00 3.04 2.91 | 0.151 .570 .372 <.001 .199 |
| Day 21 | 4.44 4.08 4.40 4.46 4.16 | 0.224 |
| Day 28 | 6.43 6.00 6.33 6.39 6.11 | 0.417 |
| Body weight gains, g/day |        |
| Days 7–14 | 144 104 125 128 122 | 15.2 |
| Days 14–21 | 177 183 200 197 176 | 21.2 .906 .723 <.001 .547 |
| Days 21–28 | 251 263 252 251 260 | 29.9 |
| Dry matter intake, g/day |        |
| Days 7–14 | 289 236 252 – – | 19.0 |
| Days 14–21 | 429 362 469 – – | 36.0 .308 – <.001 .357 |
| Days 21–28 | 654 649 756 – – | 67.4 |
| Gain-to-feed ratio, g/g |        |
| Days 7–14 | 1.08 0.86 0.98 – – | 0.088 |
| Days 14–21 | 0.89 1.01 0.85 – – | 0.081 .241 – <.001 .320 |
| Days 21–28 | 0.80 0.81 0.66 – – | 0.044 |

\(^a\)Results are presented as least squares means of the main factors dietary treatment and sex and pooled standard error of the means (SEM).

\(^b\)CON = control (unsupplemented), CAR = 0.48 g \( l \)-carnitine·piglet\(^{-1}\)·day\(^{-1}\), ARG = 1.20 g \( l \)-arginine HCl·kg BW\(^{-1}\)·day\(^{-1}\).

\(^c\)Probability values for the effects of milk replacer supplementation (Trt), sex (S), time (T), and Trt × T interaction.
TABLE 3  Morphometric muscle measurements, organ and carcass weights as well as carcass composition of early-weaned piglets born from hyperprolific sows with ad libitum access to an unsupplemented milk replacer or the same milk replacer supplemented with either L-carnitine or L-arginine from day 7 to day 28 of age

| Item                        | Dietary treatmentb | Sex          | p-valuec |
|-----------------------------|-------------------|--------------|----------|
|                            | CON   | CAR   | ARG   | Male | Female | SEM | Trt | Sex | Trt × Sex |
| Number                      | 12    | 12    | 12    | 18   | 18     |     |     |     |          |
| Semitendinosus muscle       |       |       |       |       |        |     |     |     |          |
| Weight, g                   | 17.8  | 17.8  | 18.2  | 17.5 | 18.3   | 1.91| .989| .629| .898     |
| Length, mm                  | 68.4  | 68.9  | 70.8  | 69.6 | 69.4   | 3.11| .878| .876| .927     |
| Circumference, mm           | 70.7  | 73.0  | 71.9  | 71.5 | 72.2   | 3.51| .828| .803| .596     |
| Organ weight, g             |       |       |       |       |        |     |     |     |          |
| Heart                       | 33.4  | 37.4  | 36.2  | 36.8 | 34.6   | 2.68| .457| .348| .813     |
| Liver                       | 198   | 179   | 205   | 188  | 200    | 16.7| .563| .510| .934     |
| Kidneys                     | 45.6  | 44.4  | 41.2  | 43.5 | 44.0   | 4.10| .777| .876| .782     |
| Spleen                      | 9.30  | 9.11  | 9.97  | 10.02| 8.90   | 1.080| .876| .227| .797     |
| Lung                        | 76.6  | 79.0  | 78.8  | 78.2 | 78.1   | 6.77 | .957| .979| .867     |
| Adrenal glands              | 0.76  | 0.73  | 0.85  | 0.78 | 0.77   | 0.059| .437| .834| .496     |
| Brain                       | 50.3  | 47.5  | 49.7  | 48.9 | 49.4   | 1.23 | .185| .618| .389     |
| Left carcass weight, kg     | 2.23  | 2.14  | 2.13  | 2.21 | 2.12   | 0.155| .892| .563| .749     |
| Carcass composition, per kg |       |       |       |       |        |     |     |     |          |
| Gross energy, MJ            | 24.2  | 23.9  | 24.2  | 24.1 | 24.1   | 0.39 | .823| .954| .255     |
| Crude protein, g            | 580   | 588   | 584   | 585  | 582    | 11.6 | .867| .756| .884     |
| Ether extract, g            | 262   | 254   | 268   | 258  | 265    | 15.6 | .838| .637| .732     |
| Total ash, g                | 142   | 140   | 134   | 140  | 134    | 5.6  | .643| .408| .168     |

aResults are presented as least squares means of the main factors dietary treatment and sex and pooled standard error of the means (SEM).
bCON = control (unsupplemented), CAR = 0.48 g L-carnitine-piglet⁻¹·day⁻¹, ARG = 1.20 g L-arginine HCl·kg BW⁻¹·day⁻¹.
cProbability values for the effect of milk replacer supplementation (Trt), sex (S) and Trt × S interaction.

males, females), day or week, and the two- and three-way interactions were included as fixed effects. Litter, nested within farrowing series, was considered as random effect accounting for the dependence between observations within the same litter and animal was used as the experimental unit. As the dietary treatment × sex, sex × day/week and dietary treatment × sex × day/week interactions were never significant probability values of these effects were not presented. For the analysis of the data on feed intake and gain-to-feed ratio, determined per pen, the factor sex was omitted providing dietary treatment, week and the two-way interaction as fixed effects, farrowing series as random effect and pen as the experimental unit. Data on carcass and muscle weight were analysed considering dietary treatment, sex and the dietary treatment × sex interaction as fixed effects, litter nested within farrowing series as random effect and animal as the experimental unit. Data on muscle histology, enzyme activity, phosphorylation of the mTOR pathway and gene expression additionally considered STM portion and corresponding two- and three-way interactions as fixed effects. As the effect of dietary treatment × sex, sex × muscle portion and dietary treatment × sex × muscle portion interactions were rarely significant, these least square means were only reported in the text when p < .10 and probability values were not presented. The PDIFF option with the Tukey adjustment was used to determine differences among treatment groups. Differences were considered statistically significant at p < .05, and tendencies were assumed at .05 < p < .10.

3 | RESULTS

No difference between the later dietary treatment groups was observed with respect to BtW (CON = 1.180 ± 0.108; CAR = 1.179 ± 0.134; ARG = 1.179 ± 0.198 kg) and ADG from birth to day 7 (CON = 155 ± 38; CAR = 141 ± 42; ARG = 156 ± 40 g/day) resulting in a similar BW on day 7 (Table 2). The treatment had no effect on BW, ADG, feed intake and gain-to-feed ratio in any of the weekly assessments. In contrast, measurement day or week (time) had an effect (p < .05) on these four traits as BW, ADG and feed intake increased and gain-to-feed ratio decreased with time. There was no significant dietary treatment × time
interaction. Dietary treatment had also no effects on muscle morphometric traits, organ weights and carcass composition (Table 3).

Within STM_d and STM_l cross-sectional area and distribution of type I, Ila and IIb myofibres were not affected by the dietary treatments (Table 4). In the STM_d compared with the STM_l, type I and Ila myofibres were larger (p = 0.026), type IIb myofibres tended (p = 0.080) to be larger, and area and number percentage of type I and type Ila was greater (p < 0.001) and of type IIb lower (p < 0.001). Furthermore, the proportion of type Ila myofibre area tended (p < 0.10) to be affected by the dietary treatment; however, Tukey’s post hoc multiple comparison test indicated that there were no difference between the three groups. Regarding type I area percentage, there was an effect (p = 0.05) of sex (male vs. female; 15.7 vs. 12.6%) in the STM_d. Furthermore, males compared with females displayed greater type I myofibre area percentage (29.1 vs. 22.9%; sex × muscle portion interaction; p < 0.05) also in the STM_d. The total number of myofibres in the STM was similar among treatments (CON = 919 × 10^3; CAR = 990 × 10^3; ARG = 922 × 10^3 [SEM = 69.7 × 10^3]). Also the relative expression of the five MyHC isoforms differed mainly among the muscle portions (Table 4). In the STM_d of CON and ARG piglets, MyHC IIB expression tended to be lower in CON compared with ARG, whereas no treatment differences were observed in the STM_l. Compared with the STM_l, the expression of the embryonic, IIX and IIB MyHC isoforms was lower (p ≤ 0.004) and that of MyHC I and IIA was greater (p < 0.001) compared with the STMl. The MyHC IIX isoform expression in the STM_d tended to be lower in CON compared with CAR and with intermediate values for ARG (dietary treatment × muscle portion interaction; p = 0.097), whereas no treatment differences were observed in the STM_l. Compared with the STM_l, the MyHC IIB gene expression tended to be lower in the STM_d of CON and ARG piglets, but not in that of CAR piglets (dietary treatment × muscle portion interaction; p = 0.077). Gene expression of MyHC I was greater in females than in males of the CAR group (1.56 vs. 0.56; dietary treatment ×

### TABLE 4 Cross-sectional area and relative distribution (expressed as number and area percentage) of type I, Ila and IIb myofibres and relative expression of myosin heavy chains isoform (MyHC) genes in the dark (STM_d) and light (STM_l) portion of the semitendinosus muscle of early-weaned piglets born from hyperprolific sows with ad libitum access to an unsupplemented milk replacer or the same milk replacer supplemented with either L-carnitine or L-arginine from day 7 to day 28 of age.

| Item                        | STM_d b | STM_l c | p-value c |
|-----------------------------|---------|---------|-----------|
|                             | CON     | CAR     | ARG       | SEM  | Trt | S   | M | Trt × M |
| Number                      | 12      | 12      | 12        |      |     |     |   |         |
| Cross-sectional area of myofibres, μm² |         |         |           |      |     |     |   |         |
| Type I                      | 521     | 561     | 518       | 24.03| 26.61| 27.37|    |         |
| Type Ila                    | 565     | 564     | 526       | 43.04| 45.41| 46.42|    |         |
| Type IIb                    | 659     | 643     | 592       | 32.67| 28.06| 26.22|    |         |
| Area distribution, %d       |         |         |           |      |     |     |   |         |
| Type I                      | 24.03   | 26.61   | 27.37     | 1.98 | 2.47 | 2.20 | 1.596| .485   | .020  | <.001 | .612  |
| Type Ila                    | 43.04   | 45.41   | 46.42     | 29.46| 36.55| 34.40| 2.652| .159   | .725  | <.001 | .664  |
| Type IIb                    | 32.67   | 28.06   | 26.22     | 68.30| 61.07| 63.41| 2.888| .093   | .320  | <.001 | .747  |
| Number distribution, %e     |         |         |           |      |     |     |   |         |
| Type I                      | 26.73   | 26.51   | 28.45     | 2.47 | 2.80 | 2.85 | 1.646| .771   | .119  | <.001 | .842  |
| Type Ila                    | 44.49   | 47.42   | 47.58     | 33.90| 39.57| 36.29| 2.947| .346   | .824  | <.001 | .818  |
| Type IIb                    | 28.49   | 26.06   | 24.03     | 63.34| 57.62| 60.91| 2.932| .336   | .506  | <.001 | .610  |
| Gene expression analysisf   |         |         |           |      |     |     |   |         |
| MyHC emb                    | 1.56    | 1.56    | 1.32      | 3.51 | 2.82 | 1.82 | 0.545| .238   | .432  | <.004 | .365  |
| MyHC I                      | 1.36    | 1.62    | 1.54      | 0.35 | 0.51 | 0.35 | 0.254| .716   | .147  | <.001 | .934  |
| MyHC IIA                    | 1.10    | 1.38    | 1.23      | 0.81 | 0.61 | 0.65 | 0.189| .958   | .725  | <.001 | .389  |
| MyHC IIX                    | 0.46    | 1.08    | 0.78      | 1.30 | 1.31 | 1.15 | 0.213| .205   | .222  | <.001 | .097  |
| MyHC IIB                    | 0.53    | 0.69    | 0.27      | 2.37 | 1.50 | 2.35 | 0.326| .546   | .493  | <.001 | .077  |

Within row, least squares means without a common superscript differ (5 < p < .10).

Con = control (unsupplemented), CAR = 0.48 g L-carnitine-piglet⁻¹·day⁻¹, ARG = 1.20 g L-arginine HCl·kg BW⁻¹·day⁻¹.

Probability values for the effects of milk replacer supplementation (Trt), sex (S), muscle portion (M) and Trt × M interaction.

Area distribution is expressed as the percentage of the summed area of the individual myofibre type relative to the summed area of all three myofibre types in a defined area of 0.14 mm² of the STM_d and STM_l.

Number distribution is expressed as the percentage of the summed number of the individual myofibre type relative to the summed number of all three myofibre types in a defined area of 0.14 mm² of the STM_d and STM_l.

MyHC emb = Embryonic myosin heavy chain, MyHC I = Myosin heavy chain I, MyHC IIA = Myosin heavy chain IIA, MyHC IIX = Myosin heavy chain IIX, MyHC IIB = Myosin heavy chain IIB.
TABLE 5 Enzyme activities characterizing oxidative (citrate synthase, CS), glycolytic capacity (lactate dehydrogenase, LDH), lipid oxidation (β-hydroxyacyl-CoA dehydrogenase, HAD), the relative importance of lipid oxidation over total oxidative capacity (HAD:CS), and the relative importance of glycolytic over lipid oxidation (LDH:HAD) and total oxidative (LDH:CS) capacity in the dark (STMd) and light (STMl) portion of the semitendinosus muscle of early-weaned piglets born from hyperprolific sows with ad libitum access to an unsupplemented milk replacer or the same milk replacer supplemented with either l-carnitine or l-arginine from day 7 to day 28 of age.

| Item                          | STMd b | STMl | p-value c |
|-------------------------------|--------|------|-----------|
|                               | CON    | CAR  | ARG       | SEM | Trt  | S   | M    | Trt × M |
| Number                        | 12     | 12   | 12        |     |      |     |      |         |
| Enzyme activity, μM/min       |        |      |           |     |      |     |      |         |
| HAD                           | 0.279y | 0.365z | 0.351yz  | 0.170w | 0.238x | 0.197w | 0.0341 | .085 | .043 | <.001 | .780 |
| CS (× 10−2)                   | 0.346  | 0.307 | 0.303     | 0.226 | 0.278 | 0.227 | 0.0390 | .823 | .070 | .002 | .265 |
| LDH                           | 2.41   | 2.97  | 2.68      | 3.29 | 3.76  | 2.93  | 0.530  | .450 | .412 | .025 | .612 |
| HAD:CS                        | 88.8   | 148.7 | 127.9     | 84.1 | 111.2 | 101.5 | 20.7   | .110 | .612 | .136 | .667 |
| LDH:HAD                       | 10.4   | 10.3  | 7.7       | 20.1 | 26.1  | 16.1  | 5.20   | .546 | .602 | .003 | .680 |
| LDH:CS                        | 647    | 1507  | 947       | 1468 | 1927  | 1375  | 344.3  | .135 | .034 | .031 | .758 |

a,b,c Within row, least squares means without a common superscript differ (0.05 < p < 0.10).

aResults are presented as least squares interaction means of the main factor dietary treatment × muscle portion type and pooled standard error of the means (SEM).

bCON = control (unsupplemented); CAR = 0.48 g l-carnitine-piglet−1·day−1; ARG = 1.20 g l-arginine HCl·kg BW−1·day−1.

cProbability values for the effects of milk replacer supplementation (Trt), sex (S), muscle portion (M) and Trt × M interaction.

sex interaction; p = .043), whereas no sex difference was found in the CON and ARG treatments. Except for a tendency (p < .10) towards an increased HAD activity in CAR compared with CON, but not ARG piglets, oxidative (CS) and glycolytic (LDH) capacity of the STMd and STMl did not differ among dietary treatments (Table 5). The trend for a difference in HAD activity was manifested in greater values in CAR males than CAR females (0.374 vs. 0.229 μM/min; dietary treatment × sex interaction effect; p < .10). There was no dietary treatment difference in the LDH:HAD and LDH:CS ratios. As expected, the HAD and CS activities were greater (p = .070) in the STMd compared with the STMl. In accordance, the relative importance of glycolytic over lipid oxidation (LDH:HAD) and overall oxidative capacity (LDH:CS) was lower (p = .031) in the STMd compared with the STMl. The HAD and CS activities were greater (p = .070) and relative importance of glycolytic over oxidative metabolism (LDH:CS) was lower (p = .034) in males than females (HAD: 0.294 vs. 0.239 μM/min; CS: 0.303 vs. 0.259 μM/min; LDH:CS: 1032 vs. 1592 μM/min). There was a sex × muscle portion interaction in HAD activity (p < .05) (male vs. female in STMd: 0.387 vs. 0.276 μM/min; p = .024; STMl vs. STMl in male: 0.387 vs. 0.201 μM/min; p < .001) and HAD:CS ratio (p < .05) (STMd vs. STMl in male; 133 vs. 79; p = .068) was observed, indicating that the degree of lipid oxidation is muscle portion specific in males but not females.

Dietary supplements had an effect (p < .05) on mTOR phosphorylation on the Ser2481 residue with greater (p < .05) degree of phosphorylation observed in CAR compared with CON, but not ARG in the STMl but not STMd (dietary treatment × muscle portion interaction; p < .05; Figures 1 and 2). The expression level of the RPS6KB1 gene

FIGURE 1 Effect of l-carnitine (CAR) (n=12); light grey bar) or l-arginine (ARG) (n=12); dark grey bar) compared with (CON) (n=12); white bar) on the degree of phosphorylation of residue Ser2481 of the mammalian target of rapamycin pathway in the dark (STM dark) and light (STM light) portion of the semitendinosus muscle. Results are presented as arbitrary units, where each bar represents the least square mean of each dietary treatment ± SE, and between bars degree of phosphorylation without a common letter differ (p < .05)

FIGURE 2 Western blot of the phosphorylated residue Ser2481 of the mammalian target of rapamycin pathway. Bands of a reference sample (Ref) and a sample from the semitendinosus muscle of one control (CON), l-carnitine (CAR) and l-arginine (ARG) piglet each is presented.
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4 | DISCUSSION

In recent years, the novel measure of artificial rearing of piglets in rescue decks has been introduced and investigated in its suitability to improve survival rate of the increased number of growth-impaired LW piglets from hyperprolific sows (De Vos, Huygelen, et al., 2014; Rzezniczek, Gygax, Wechsler, & Weber, 2015). In alternative rearing strategies such as split-nursing, it is common practice that the largest piglets of the litters are transferred to nursing sows, while the smaller piglets remain with the sow of origin (De Vos, Che, et al., 2014). However, as LW growth-impaired piglets appear to be less mature and therefore less vital (Rutherford et al., 2013), and are prone to get crushed below the sow due to severe starvation (Edwards, 2002), it is plausible that rearing these piglets in rescue decks will increase the overall survival rate of the litter and ultimately improve production efficiency. In the present study, only one piglet deceased during the trials, which was later replaced to achieve the desired total number of 36 animals. In comparison to previously reported results of conventionally reared piglets, the mortality rate in this study was similar to lower between day 7 and day 28 (2.7% vs. 1.6%-5.0% [Hales, Moustsen, Nielsen, & Hansen, 2014]), where the main course of death is reported to be crushing by the sow (Edwards, 2002). In a previous study conducted at the same research station, a very similar approach with artificial rearing was used, but in contrast restricted provision of supplemented milk replacer was applied which was not able to promote growth (Madsen et al., 2014). Therefore, in the present study ad libitum provision of milk replacer was applied expecting that this would lead to a greater feed intake resulting in a greater growth response of LW piglets and possibly to a greater impact of L-carnitine and L-arginine.

4.1 | Effect of arginine and carnitine, as well as artificial rearing on growth performance

Harrell et al. (1993) estimated that pre-weaning growth is limited by at least 40%, due to inadequate secretion of sow milk. In addition, it is also evident that homemade milk replacers previously used in research (Kim & Wu, 2004; Yao et al., 2008) are suboptimal when comparing fat, protein and energy content and amino acid composition of the one used in the present study with the composition of the sow milk analysed (Table 1). Thus, when daily amount of milk consumed does not differ between artificially reared and suckling piglets, it would naturally result in greater ADG of suckling piglets (Theil et al., 2007). However, despite the suboptimal milk replacer diet applied in this study, artificial rearing resulted in similar weaning weight when compared with conventionally reared piglets with similar birthweight (6.3 vs. 6.7 kg). This was improved substantially compared with our previous effort with weaning weights <5.0 kg where a restricted feeding regime was applied (Madsen et al., 2014). This indicates that the capacity of LW piglets to ingest feed during the nursing period exceeds the previously established assumed requirements for daily intake (Kim & Wu, 2004). In addition, the weaning weight recorded in the present study was comparable to a recent and similar study, where artificially reared LW piglets reached a BW of 6.2 kg at day 28 of age (De Vos, Huygelen, et al., 2014). Still, the positive impact of rescue decks on growth and, thus assumed better welfare, has been questioned by a recent study comparing behaviour between piglets reared in rescue decks and piglets reared by the sow in a loose housed sow system (Rzezniczek et al., 2015).

Elevating the content of L-arginine from 7 to 11 g/kg in a milk replacer positively affected growth of nursing piglets in the study of Kim and Wu (2004). In the present experiment, only a numerical improvement of day-28 BW by ARG compared with CAR but not CON piglets was observed. This lack of clear effect could have been due to a suboptimal arginine:lysine ratio, leading to an overall imbalanced amino acid composition which would limit growth performance (D’Mello, 2003). As shown in two previous studies providing weaners with a diet containing 16 g arginine per kg dry matter even decreased feed intake and ADG (Anderson, Lewis, Peo, & Crenshaw, 1984), and an increase in arginine from 6.7 to 20.0 g/kg of the diet reduced daily growth (Southern & Baker, 1982). However, in both studies increased levels of lysine did not ameliorate the adverse effects. In a more recent effort, Gette, Almeida, Baratta, and Dilger (2015) showed that L-arginine supplemented to conventionally reared LW piglets also lead to reduced growth. In all three studies it was concluded that the impaired performance was caused by a
general amino acid imbalance rather than arginine–lysine antagonism. In the present study the content of arginine in the ARG diet was 18.7 g/kg accounting for arginine present in the basic milk replacer and the supplement. This was associated with a numerical lower BW on day 28 compared with the CON piglets, and reduced gain-to-feed ratio compared with the CON and CAR group from day 21 to day 28. However, more importantly in the light of the aforementioned amino acid imbalance, a less than optimal arginine:lysine ratio in the milk replacer (1.10) was found when compared with the results of Kim and Wu (2004) (0.48). Thus, it appeared that the l-arginine recommendations by Wu et al. (2000) for young piglets of 1.08 g kg⁻¹-BW⁻¹-day⁻¹ did not correspond to the requirements of LW piglets from hyperprolific Swiss Large White sows and were not established relative to the lysine content. Regarding CAR piglets, growth performance did not differ from the CON group at all. This was expected as this supplement was included to promote post-natal hyperplasia and not growth performance as shown by Lösel et al. (2009).

4.2 | Effect of arginine and carnitine on myofibre properties and activation of muscle protein synthesis

It has been shown that a daily oral dosage of l-carnitine given to conventionally reared LW piglets promoted post-natal hyperplasia in the suckling period (Lösel et al., 2009). However, recently the same research group did not find any difference in TNF in the STM of pigs slaughtered at day 166 of age, which received daily either a placebo or an oral dosage of l-carnitine in the nursing period (Lösel & Rehfeldt, 2013). The discrepancy between the results on TNF obtained at weaning and at slaughter was to their mind explained either by a greater TNF at weaning as a consequence of an actual third wave of hyperplasia or by an elongation of already existing intrafascicularly terminating myofibres. The latter might also explain the numerical difference of TNF between dietary treatments found in the present study. It has been suggested that the embryonic MyHC isoform is a biomarker for post-natal hyperplasia showing peak upregulation at day 63 of gestation (Da Costa, McGillivray, & Chang, 2003), the time where secondary myofibre formation is completed (Swatland, 1973). However, embryonic MyHC isoform gene was still expressed at day 28 in all three dietary groups, although as discussed in Bérard, Kalbe, Lösel, Tuchscherer, and Rehfeldt (2011) contradictory opinions exist regarding whether this expression is due to the regeneration of existing or the formation of tertiary myofibres. Also noteworthy regarding the embryonic MyHC isoform is its greater expression in STM compared with the STMg, indicating not only the only obvious difference in proportion of myofibre types and metabolism between muscle portions, but possibly also difference in speed of development as observed between different muscles showing different disappearance rates of this particularly MyHC isoform (Gambke & Rubinstein, 1984).

Although no difference in myofibre hypertrophy or muscle weight was found between dietary treatments, some of the present results suggest the presence of an induced activation of the protein synthesis pathway. Mammalian target of rapamycin is a member of the phosphoinositide 3-kinase-related kinase family, and is a signalling pathway involved in coordinating cell growth and division in response to growth factors, nutrient and energy status of the cell (Wang & Proud, 2006). Upon activation, mTOR can be phosphorylated on multiple residues including Thr²⁴⁴⁶, Ser²⁴⁸⁶, Ser¹²⁶¹ and Ser²⁴⁸¹ (Foster & Fingar, 2010). In the present study, the latter was investigated for its extent of phosphorylation. Among other pathways, mTOR is essential in muscle tissue catabolism. In the present study, a numerically increased mTOR phosphorylation was observed in the STM, of ARG relative to CON piglets. This observation is to some extent in agreement with a previous study, which showed that an elevated level of l-arginine resulted in increased phosphorylation of mTOR on residue Ser²⁴⁸⁶, and improved growth performance of early-weaned milk replacer fed piglets (Yao et al., 2008). Furthermore, also l-carnitine was found to elicit an enhanced mTOR phosphorylation on Ser²⁴⁸¹ in the STM, similar to an effect observed in rat muscle (Keller, Couturier, Haferkamp, Most, & Eder, 2013). In that, and in a previous study investigating l-carnitine supplementation of pigs, no changes in BW nor muscle weight were observed (Keller et al., 2012). In contrast, l-carnitine has in several species including pigs, chickens and rats been proposed to increase protein accretion through increasing circulating levels of insulin-like growth factor 1 (IGF-1), an anabolic hormone indirectly involved in mTOR activation (Ringseis, Keller, & Eder, 2013). On the molecular level, the expression of the RPS6KB1 gene was numerically greater in the STM of the present ARG group compared with CAR piglets. This gene encodes the S6K1 kinase enzyme and has a functioning role in the mTOR signalling pathway acting downstream of mTOR (Wang & Proud, 2006), and it is directly involved in protein synthesis in muscle (Kimball et al., 2000). Thus, this might link the observation of a numerical increased phosphorylation of mTOR with increased RPS6KB1 expression in the STM of the present ARG piglets.

4.3 | Effect of arginine and carnitine on the maturation process of the muscle

l-arginine and l-carnitine supplementation have separately shown to affect muscle maturation by a premature shift from oxidative to glycolytic metabolism in STM of early-weaned LW piglets (Madsen, Kreuzer, & Bee, 2016). A similar finding was reported in LW piglets (< 1.22 kg BtW) where l-arginine supplementation (0.48 g kg⁻¹ BW⁻¹-day⁻¹) slightly stimulated the metabolic maturation in the STM, as indicated by increases in activities of creatine kinase and LDH. In the present study only numerical indications were found for possible earlier muscle maturation in ARG- and CAR-supplemented piglets. Although not related to muscle maturation, the enhanced HAD activity found in the STM of CAR compared with CON piglets is consistent with the function of l-carnitine acting as a transporter of long chain fatty acids into the mitochondria for energy production via beta-oxidation, where HAD acts as a catalyst (Wakil, Green, Mill, & Mahler, 1954). In contrast to that, on the molecular level results from the present study suggest that the individual supplements act differently in the two STM portions, displaying different mechanistic properties related to the expression of MyHC isoform genes. Increasing age and muscle maturity is in general associated with a shift towards fast
glycolytic metabolism with the dominating myofibre types being type IIX and IIB (Chang & Fernandes, 1997). Surprisingly, in the STMd gene expression of MyHC IIX isoform was greater than that of the MyHC IIB isoform. MyHC IIX is expressed in oxido-glycolytic myofibres and regarded as an intermediate myofibre type between type IIA and IIB in terms of contractile properties. Thus, given the transition order of the MyHC isoforms following the pathway from MyHC I ↔ IIA ↔ IIX ↔ IIB with increasing age and dependent on muscle functionality (Pette & Staron, 2000), it appears that in the present study l-carnitine promoted maturation in the STMd. Although not displaying significant differences, LDH and LDH:CS ratio were numerically greater in the STMd of CAR than CON pigs.

A sex effect and dietary treatment × sex interactions were found concerning the metabolic properties and at the gene expression level of the muscle. Most interestingly, males compared with females, were more affected by l-carnitine in the STMd in its target pathway catalysed by HAD. Furthermore, males appeared to rely more on the oxidative than the glycolytic metabolism in the STMl compared with females. The lower LDH:CS ratio, an indicator of muscle maturity (Lefaucheur, Ecolan, Barzic, Marion, & Le Dividich, 2003), suggests that at day 28 of age, the muscles of the males were less mature than those of the females. In addition, on the molecular level the potential for protein synthesis appeared to be more pronounced in males supported by the greater expression of RPS6KB1.

5 | CONCLUSION

In conclusion, there were a few responses in indicators of protein synthesis of the lightweight piglets from large litters to l-carnitine (enhanced mTOR phosphorylation in the STMd and l-arginine (expression of a gene involved in protein synthesis in STMd). However, different from previous studies, the concentrations of l-arginine and l-carnitine chosen to be supplemented to a quite common type of milk replacer did not elicit the expected effects on growth performance of early-weaned LW piglets even when offered at ad libitum access. One explanation for the lacking effect could be that the requirements are not up to date with current genotypes of LW piglets from hyperprolific sows. Another and more likely explanation is that the milk replacer was not only lacking arginine, but possibly also other amino acids like lysine in comparison with sow milk. Thus, the approach of only supplementing arginine would lead to an amino acid imbalance. Further research should focus on a more holistic strategy concerning the entire amino acid composition of milk replacers. The high survival rate observed in this study suggests that applying rescue decks can be an important measure to reduce losses of piglets from large litters during the mid to late nursery phase.

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REFERENCES

Andersen, I. L., Naevdal, E., & Boe, K. E. (2011). Maternal investment, sibling competition, and offspring survival with increasing litter size and parity in pigs (Sus scrofa). Behavioral Ecology and Sociobiology, 65, 1159–1167.
Anderson, L. C., Lewis, A. J., Peo, E. R., & Crenshaw, J. D. (1984). Effects of excess arginine with and without supplemental lysine on performance, plasma amino acid concentrations and nitrogen balance of young swine. Journal of Animal Science, 58, 369–377.
AOAC (Association of Official Analytical Chemists) (2012). Official methods of analysis, 19th edn. Gaithersburg, MD, USA: AOAC. 15 edn. Association of Official Chemists, Washington, DC.
Bérard, J., & Bee, G. (2010). Effects of dietary l-arginine supplementation to gilts during early gestation on foetal survival, growth and myofiber formation. Animal, 4, 1680–1687.
Bérard, J., Kalfé, C., Lüscher, D., Tuchschzercher, A., & Rehfeldt, C. (2011). Potential sources of early-postnatal increase in myofibre number in pig skeletal muscle. Histochemistry and Cell Biology, 136, 217–225.
Bérard, J., Pardo, C. E., Bethaz, S., Kreuzer, M., & Bee, G. (2010). Intrauterine crowding decreases average birth weight and affects muscle fiber hyperplasia in piglets. Journal of Animal Science, 88, 3242–3250.
Chang, K. C., & Fernandes, K. (1997). Developmental expression and S’ end cDNA cloning of the porcine 2x and 2b myosin heavy chain genes. DNA Cell and Biology, 16, 1429–1437.
Da Costa, N., McGillivray, C., & Chang, K. C. (2003). Postnatal myosin heavy chain isoforms in prenatal porcine skeletal muscles: Insights into temporal regulation. The Anatomical Record, 273A, 731–740.
De Vos, M., Che, L., Huygelen, V., Willemen, S., Michielis, J., Van Cruchten, S., & Van Ginneken, C. (2014). Nutritional interventions to prevent and rear low-birthweight piglets. Journal of Animal Physiology and Animal Nutrition, 98, 609–619.
De Vos, M., Huygelen, V., Willemen, S., Fransen, E., Casteleyn, C., Van Cruchten, S., Van Ginneken, C. (2014). Artificial rearing of piglets: Effects on small intestinal morphology and digestion capacity. Livestock Science, 159, 165–173.
Deen, M. G. H., & Bilkei, G. (2004). Cross fostering of low-birthweight piglets. Livestock Production Science, 90, 279–284.
D’Mello, J. P. F. (2003). Adverse effects of amino acids. In J. P. F. D’Mello (Ed.), Amino acids in animal nutrition (pp. 125–142). UK: CABI publishing, Wallingford.
Edwards, S. A. (2002). Perinatal mortality in the pig: Environmental or physiological solutions? Livestock Production Science, 78, 3–12.
Foster, K. G., & Fingar, D. C. (2010). Mammalian Target of Rapamycin (mTOR): Conducting the cellular signaling symphony. The Journal of Biological Chemistry, 285, 14071–14077.
Gambke, B., & Rubinstein, N. A. (1984). A monoclonal antibody to the embryonic myosin heavy chain of rat skeletal muscle. The Journal of Biological Chemistry, 259, 12092–12100.
Getty, C. M., Almeida, F. N., Baratta, A. A., & Dilger, R. N. (2015). Plasma metabolomics indicates metabolic perturbations in low birth weight piglets supplemented with arginine. Journal of Animal Science, 93, 5754–5763.
Hales, J., Moustsen, V. A., Nielsen, M. B. F., & Hansen, C. F. (2014). Higher preweaning mortality in free farrowing pens compared with farrowing crates in three commercial pig farms. Animal, 8, 113–120.
Harrell, R. J., Thomas, M. J., & Boyd, R. D. (1993). Limitations of sow milk yield on baby pig growth. Proceedings Cornell Nutrition Conference (pp. 156–164), Ithaca, NY.
ISO (International Organization for Standardization) (1999). ISO 6492:1999. Animal feeding stuffs - Determination of fat content. ISO, Geneva, Switzerland.
Madsen, J. G., Kreuzer, M., & Bee, G. (2016). ECO FCE: Peri-natal nutrition and lifetime performance of low birth weight pigs from prolific sows. Animal Welfare, 25, 134–138.

Keller, J., Ringseis, R., Koc, A., Lukas, I., Kluge, H., & Eder, K. (2012). Supplementation with l-carnitine downregulates genes of the ubiquitin proteasome system in the skeletal muscle and liver of piglets. Animal, 6, 70–78.

Kim, S. W., & Wu, G. (2004). Dietary arginine supplementation enhances the growth of milk-fed young pigs. Journal of Nutrition, 134, 625–630.

Kimball, S. R., Jefferson, L. S., Nguyen, H. V., Suryawan, A., Bush, J. A., & Mavromichalis, I., Parr, T. M., Gabert, V. M., & Baker, D. H. (2001). True ileal digestibility of amino acids in sow’s milk for 17-day-old pigs. Journal of Animal Science, 79, 707–713.

Pette, D., & Staron, R. S. (2000). Myosin isoforms, muscle fiber types, and transitions. Microscopy Research and Technique, 50, 500–509.

Quiniou, N., Dagorn, J., & Gaudré, D. (2002). Variation of piglets’ birth weight and consequences on subsequent performance. Livestock Production Science, 78, 63–70.

Rutherford, K. M. D., Baxter, E. M., D’Eath, R. B., Turner, S. P., Arnott, G., Roche, R., ... Lawrence, A. B. (2013). The welfare implications of large litter size in the domestic pig I: Biological factors. Animal Welfare, 22, 199–218.

Rzeznicek, M., Gygax, L., Wechsler, B., & Weber, R. (2015). Comparison of the behaviour of piglets raised in an artificial rearing system or reared by the sow. Applied Animal Behaviour Science, 165, 57–65.

Southern, L. L., & Baker, D. H. (1982). Performance and concentration of amino acids in plasma and urine of young pigs fed diets with excesses of either arginine or lysine. Journal of Animal Science, 55, 857–866.

Swatland, H. J. (1973). Muscle growth in the fetal and neonatal pig. Journal of Animal Science, 37, 536–545.

Theil, R. K., Kristensen, N. B., Jorgensen, H., Labouriau, R., & Jakobsen, K. (2007). Milk intake and carbon dioxide production of piglets determined with the doubly labelled water technique. Animal, 1, 881–888.

VDLUFA (1988). Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten. In English: Association of German agricultural analytic and research institutes. 5.1.1 Bestimmung von Rohfett, 2. Erg. In C. Nauman, R. Bassler, R. Seibold & C. Barth (Eds.), Band III Die chemische Untersuchung von Futtermitteln, 3rd edn (pp. 1–8). Germany: Speyer.

Wakil, S. J., Green, D. E., Mil, S., & Mahler, H. R. (1954). Studies on the fatty acid oxidizing system of animal tissues. VI. Beta-hydroxyacyl coenzyme A dehydrogenase. Journal of Biological Chemistry, 207, 631–638.

Wang, X., & Proud, C. G. (2006). The mTOR pathway in the control of protein synthesis. Physiology, 21, 362–369.

Weary, D. M., Pajor, E. A., Thompson, B. K., & Fraser, D. (1996). Risky behaviour by piglets: A trade off between feeding and risk of mortality by maternal crushing? Animal Behaviour, 51, 619–624.

Wu, G., Meininger, C. J., Knabe, D. A., Bazer, F. W., & Rhoads, J. M. (2000). Arginine nutrition in development, health and disease. Current Opinion in Clinical Nutrition and Metabolic Care, 3, 59–66.

Yao, K., Yin, Y. L., Chu, W., Liu, Z., Deng, D., Li, T., ... Wu, G. (2008). Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. Journal of Nutrition, 138, 867–872.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics, 13, 134.

Zijlstra, R. T., Whang, K. Y., Easter, R. A., & Odle, J. (1996). Effect of feeding a milk replacer to early-weaned pigs on growth, body composition, and small intestinal morphology, compared with suckled littermates. Journal of Animal Science, 74, 2948–2959.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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