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# Effect of maternal feed restriction in dairy goats at different stages of gestation on skeletal muscle development and energy metabolism of kids at the time of births

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ABSTRACT

The aim was to determine effects of maternal feed restriction in dairy goats at gestational different stages on skeletal muscle development and energy metabolism in kids at birth. Six pregnant goats were fed 50% of total digestible nutrients (TDN) and crude protein (CP) (NRC, 2007) recommendations in the first half of gestation and then fed to 100% of the recommendations in the second half of gestation (treatment R-M). In the other group, eight pregnant goats were fed 100% of TDN and CP in the first half of gestation and 50% of a restricted diet the second half of gestation (treatment M-R). Birth weight, blood glucose concentration, muscle fiber number, and size of kids at birth were not affected by maternal feed restriction. The mRNA and protein abundance of myogenic, adipogenic and fibrogenic markers were not affected ($P > 0.05$) by maternal diet. With regard to values for variables in kid energy metabolism, mRNA abundance of the glycolic enzyme *HKII* was less ($P = 0.03$) in the M-R group. In conclusion, maternal feed restriction in the first or second half of gestation had no affect mRNA abundance on myogenic, adipogenic, and fibrogenic markers nor were there changes in skeletal muscle mesenchymal stem cell population of kids at the time of birth. There, however, may be detrimental effects on energy metabolism by reducing *HKII* gene expression in skeletal muscle of dairy goat kids at the time of birth.

## 1. Introduction

The constituents of skeletal muscle, such as myocytes, adipocytes, and fibroblasts originate from the same pool of mesenchymal cells. As a consequence, stimuli during gestation may affect the activation, differentiation, and determination of these cells for a specific lineage (Du et al., 2012). Results of previous studies indicate maternal undernutrition in early to mid-gestation reduces the number and composition of muscle fibers (Zhu et al., 2004, 2006), while a lack of nutrients during mid-to-late gestation affects adipogenesis and muscle fiber size (Du et al., 2010; Underwood et al., 2010).

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Besides the effect of maternal nutrition during gestation on the cell fate in the skeletal muscle, changes in skeletal muscle energy metabolism may also occur in the offspring (George et al., 2012; Aragão et al., 2014; Yang et al., 2016). There is a greater utilization of fatty acids than carbohydrates for ATP synthesis (Aragão et al., 2014) in the offspring's skeletal muscle tissue when there is restricted feed intake by dams during gestation. Because fetal skeletal muscle is also the largest insulin-sensitive tissue in the body and the primary site for insulin-stimulated glucose utilization (Beauchamp and Harper, 2016), impairment of glucose uptake and insulin signaling may lead to changes in the animal performance after birth and, thus, it is an important trait that should be investigated as a consequence of *in-utero* effects on these processes.

Even though there is considerable current knowledge about the effects of maternal nutrition on the skeletal muscle development of offspring, there is some inconsistences in finding in previous studies, possibly due to the different types and duration of feeding strategies. It has been suggested that maternal feed restriction during early gestation combined with an adequate nutrition in the remaining gestational period may lead to fetal compensatory growth (Gonzalez et al., 2013). Thus, the objective of this study was to evaluate the effects of feed restriction of does during different stages (first or second half) of gestation on skeletal muscle development and energy metabolism of dairy goat kids at birth. It, therefore, was hypothesized that feed restriction in different stages of gestation alters the differentiation of mesenchymal stem cells and energy metabolism in skeletal muscle of kids at the time of birth.

## 2. Material and methods

### 2.1. Animal husbandry

All animal care and handling procedures were approved by Animal Care and Use Committee of the Department of Animal Science at the *Universidade Federal de Viçosa, Viçosa, Minas Gerais,* Brazil (protocol 09/2017). Initially, 60 nulliparous dairy goats were submitted to estrous synchronization using prostaglandin with a 7-day interval between the first and last application and there was subsequent artificial insemination of does. All does were bred using semen from a single male, to reduce paternal effects. The day of insemination was considered as day 0 of pregnancy and the beginning of the experiment. On the same day, does were confined to individual pens (3 $m^2$), and submitted during an adaptation period of 7 days to an experimental diet and water *ad libitum*. Pregnancy was confirmed 30 days after insemination, and a total of 14 nulliparous dairy goats weighing 50 ± 13 kg and 19 ± 7 months (mean ± SD) of age had pregnancy confirmed and continued to be included as experimental animals in this study.

At the 8th day of gestation, does were randomly assigned into two treatment groups with different feeding regiments. Six pregnant goats were fed 50% of total digestible nutrients (TDN) and crude protein (CP) using the National Research Council (NRC, 2007) recommendations from day 8–84 of gestation and then does were fed to 100% of TDN and CP of NRC recommendations from day 85 of gestation until parturition (term ˜ 150 days; treatment restriction-maintenance, R−M). In the other treatment group, eight pregnant goats were fed 100% of TDN and CP of NRC recommendations from day 8–84 of gestation and then received a feed-restricted diet of 50% of TDN and CP of NRC recommendations from day 85 of gestation until parturition (treatment maintenance-restriction, M–R). Treatments consisted of the same diet for both groups with differences only in the amount of feed and gestational stage.

Animals of both groups were fed once daily (at 7:00 a.m.) and every 7 days the does were weighed in the morning before feeding. Dry matter intake and daily supply of feed were adjusted weekly based on the body weight and week of gestation of does.

Experimental diets consisted of 111.6 g/kg of crude protein and 676 g/kg of total digestible nutrients on dry matter (DM) basis and were composed of corn silage (723 g/kg DM basis), soybean meal (96 g/kg DM basis), ground corn (165 g/kg DM basis), and a mineral mixture (16 g/kg DM basis), considering the nutritional requirements for dairy goats (NRC, 2007; Table 1).

**Table 1**
Feeds and chemical composition (mean ± SEM) of the experimental diet.

| Item[a] (g/kg) | Ingredients | |
|---|---|---|
| | Corn Silage | Concentrate |
| DM[b] | 257 ± 6.93 | 845 ± 0.04 |
| OM[c] | 949 ± 2.00 | 951 ± 0.14 |
| CP[c] | 71.2 ± 1.55 | 145 ± 3.07 |
| EE[c] | 23.4 ± 1.44 | 7.00 ± 0.50 |
| NDFap[c] | 542 ± 9.21 | 147 ± 26.2 |
| NFC[c] | 313 ± 9.94 | 652 ± 29.7 |

Concentrate composition: 96 g/kg of soybean meal, 165 g/kg of ground corn, 3 g/kg of CaCO3 and 3 g/kg of CaHPO4 (DM basis).

[a] Least square means ± standard errors of: DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDFap: neutral detergent fiber corrected for ash and protein; NFC: non-fiber carbohydrate (NCF = [100-(%NDF + %CP + %EE + %Ash)].

[b] g/kg as feed.

[c] g/kg DM.

### 2.2. Feed sampling and chemical analysis

To evaluate the nutritional characteristics of the diet, samples of roughage and concentrate were collected weekly, while feed not consumed (rejected feed) was measured daily, sampled, grouped into composite samples and stored under −20 °C for further chemical analysis.

To evaluate the intake of dry matter (DMI), crude protein (CPI) and total digestible nutrient (TDNI), there was a 5-day assessment period, in the middle of each experimental period (maintenance or restriction) on days 46 and 117 of gestation, respectively. The diet provided, feed that was rejected and total feces were collected and weighed every day during the assessment period. After the assessment period, daily samples for each animal were grouped into composite samples, one for each animal.

The roughage, concentrate, feces, and rejected feed were oven-dried (55 °C/72 h), ground using a 1 mm knife in a Wiley mill (Willye® TE-680), and analyzed using the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT- CA) for dry matter (DM; INCT-CA method G-003/1), ash (INCT-CA method M-001/1), crude protein (CP; INCT-CA method N-001/1), ether extract (EE; INCT-CA method G-004/1) and neutral detergent fiber (NDFap; INCT-CA method F-002/1), corrected for ash (NDIA; INCT-CA method N-002/1) and protein (NDIP; INCT-CA method N-004/1) (Detmann et al., 2012).

### 2.3. Maternal performance

The weights corresponded with the beginning of the experiment (day 8), days on which the changes in the amount of feed provided to does occurred (day 84 and 85), and weight immediately before parturition were used to estimate maternal average daily gain (ADG) during each period. The pregnant tissues (PREG) approach (Gionbelli et al., 2015), represented by the weight related to pregnancy tissues was used to estimate the maternal and gestational ADG. The data for cattle reported by Gionbelli et al. (2015) were replaced by the goat data in the following equation as described by Castagnino et al. (2015):

$$npEBW = -7.77 + 1.03 * BW$$

where, *npEBW* is the non-pregnant empty body weight and *BW* is the body weight.

### 2.4. Tissue sampling from kids at time of birth

After birth, kids were immediately separated from the doe to avoid milk intake. This approach was imposed to avoid changes in blood glucose concentration as a result of milk intake as well as changes in transcriptome profiling of skeletal muscle tissue that might occur due to increase in glucose/insulin blood concentrations. At birth, kids were weighed, stunned using a non-penetrating captive bolt pistol, and exsanguinated. When there were twins (treatment R-M, $n = 4$; treatment M–R, $n = 5$), the heaviest kid was selected for tissue collection. Blood samples were collected at the time of exsanguination and submitted for glucose concentration analysis. After slaughter, two skeletal muscle samples were collected from the *Longissimus dorsi* muscle. One of the samples was immediately snap frozen and stored in liquid nitrogen for RNA and protein extractions. The second sample was immediately fixed in fresh formalin 10% (wt/vol) buffered to a pH of 7.4. Liver samples were collected from the tissues of each kid and immediately snap-frozen and stored in liquid nitrogen until RNA extraction was performed.

### 2.5. Skeletal muscle morphometric evaluation by histochemical and image analysis

Skeletal muscle samples, previously fixed in fresh 10% (wt/vol) formalin in phosphate buffer, were dehydrated using a crescent ethanol series and embedded in resin using the HistoResin Mounting Kit (Leica, Solmos, Hessen, Germany). Sections of 3 µm were obtained using a rotary microtome (RM 2265, Leica Biosystems, Nussloch, Germany) and stained with toluidine blue. For observation of number and diameter of muscle cells ten digital images of muscle sections per animal were obtained using a photomicroscope Olympus A×70 coupled with an AxioCam HRc- Zeiss camera at a magnification of 40x and analyzed using the ImageJ software (National Institute of Health, Baltimore, MD, USA).

To quantify the collagen content, samples were embedded in resin using HistoResin Mounting Kit (Leica, Solmos, Hessen, Germany), sections of 3 µm were cut using a microtome, and stained with Sirius-red solution (0.1% (wt/vol) of Direct Red 80 (Sigma-Aldrich) in 1.3% (wt/vol) aqueous picric acid solution) (Junqueira et al., 1979) over-night at 60 °C, washed with running water and mounted with DPX (Sigma-Aldrich). Ten digital images per animal were obtained while samples were exposed to polarized light by using a photomicroscope Olympus AX70 coupled with an AxioCam HRc- Zeiss camera at a magnification of 4x and analyzed using ImageJ software (National Institute of Health, Baltimore, MD). For quantification of the collagen content, the images were converted into grayscale, threshold to the same extent to highlight and quantify the percentage of the total image area.

### 2.6. Total RNA extraction and mRNA abundance analysis

The frozen samples were powdered in liquid nitrogen and total RNA was extracted from 0.1 g of tissue using Trizol® (InvitrogenTM, Thermo Fisher Scientific®, Oregon, USA) using the manufacturer's recommendations. Total RNA was quantified using a NanoVue spectrophotometer (GE Healthcare Life Sciences Inc.) and integrity was assessed using 1% agarose gel.

The RNA samples were then reverse transcribed into cDNA using the GoScriptTM Reverse Transcription System Kit (Promega

**Table 2**
List of primers for mRNA relative abundance analysis by RT-qPCR.

| Gene | Gene abbreviation | NCBI access code | Primer |
|---|---|---|---|
| Paired box 3 | *PAX3* | XM_005676604.3 | F: GGACTAAGAGCGAGCAAAC<br>R: CAGGTGAAGGCGAAATAGAC |
| Paired box 7 | *PAX7* | XM_018054740.1 | F: CATGCTTCCTCCAACTTCTC<br>R: AGCACATCACCACGTTTC |
| Platelet-derived growth factor receptor A, | *PDGFRα* | XM_018049462.1 | F: GGTGATGCTTTGGGAGATG<br>R:GCTCAGTCTTCACGCTTAC |
| Preadipocyte factor 1 | *PREF-1* | NM_001314212.1 | F: CATGACCACCTTCACCAAG<br>R: AACAGACCGCACAGAGA |
| Zinc finger protein 423 | *Zfp423* | XM_018061954.1 | F: GAAGAAGATGCGGGATGAC<br>R: TGGTCTTCAGGTGGATCTC |
| Peroxissome proliferator actiated-receptor gamma | *PPARγ* | NM_001285658.1 | F: GACATCGACCAACTGAACC<br>R: TCAGCGGGAAGGACTTTA |
| Collagen type I, alpha 1 | *COL1A1* | XM_018064893.1 | F: GCTTCCTGTAAACTCCTTCC<br>R: GGCTTCAGTTTGGGTTGT |
| Collagen type III, alpha 1 | *COL3A1* | XM_005675869.3 | F: AGGTGAACCCGGTAAGAA<br>R: CACCCTTAGGTCCTGGAATA |
| Hexokinase II | *HKII* | XM_018054976.1 | F: CATGATGACCTGTGGCTATG<br>R: GCGCATCTCTTCCATGTAG |
| Phosphofructokinase muscle | *PFKM* | XM_018047861.1 | F: CTGAGTGGAGTGACTTGTTG<br>R: AGGTAGCTGGACTTGGTAG |
| Pyruvate kinase muscle | *PKM* | XM_005685176.3 | F: GGGATGAAGGAGGGATACA<br>R: CTGAATCGGGTACACAAAGG |
| Insulin receptor | *INSR* | XM_018051135.1 | F: CGGACGGATTCTGACTTTG<br>R: GCCTTTGAACCAGAGAGAAG |
| Insulin receptor substrate 1 | *IRS-1* | XM_018058864.1 | F: GTCCCTCCACAGCTCTATAA<br>R: CACCTCCTCTCAGCAACTA |
| Solute carrier family 2 member 4 | *GLUT4* | NM_001314227.1 | F: CCCGCTACCTCTACATCAT<br>R: AGCCAACACCTCAGACA |
| Glucokinase (Hexokinase 4) | *HK4* | XM_013963394.2 | F: GAAGGTGATGAGGCGAATG<br>R: TAGGTGGGCAGCATCTT |
| Pyruvate kinase liver and red blood cell | *PKLR* | XM_018046254.1 | F: CTCTCAACTGGTCCCTAAGA<br>R: GAGACTGTGGCCATGATTAC |
| 18 S ribosomal | *18S* | NM_001033614 | F:CCTGCGGCTTAATTTGACTC<br>R: AACTAAGAACGGCCATGCAC |

Corporation, Madison, WI, USA), and quantified [absorbance (A) ratio at 260 and 280 nm] using a NanoVue spectrophotometer (GE Healthcare Life Sciences Inc.), with an optimal 260/280 ratio between 1.8 and 2.0. The primers (Table 2) for amplification of target and endogenous genes were designed using PrimerQuest software (www.idtdna.com/Scitools/Applications/PrimerQuest) with sequences obtained using GenBank (www.ncbi.nlm.nih.gov). Real-time quantitative PCR was performed in thermal cycler ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the detection method SYBR Green (Applied Biosystems - Foster City, CA, USA) and GoTaq® qPCR Master Mix kit (Promega Corporation, Madison, WI, USA) using the following cycle parameters: 95 °C for 2 min and 40 cycles at 95 °C for 15 s and 60 °C for 60 s. Results are expressed relative to 18S using $\ln(2^{-\Delta\Delta Ct} + 1)$ (Voge et al., 2004).

### 2.7. Protein abundance quantification using western-blot analysis

Total protein of *Longissimus dorsi* muscle was extracted from 0.1 g of powdered tissue in 1 mL of lysis buffer (10 mM Tris HCl, 100 mM of NaCl, 0.5 mM of DDT (dithiothreitol), 2.5 mM of MgCl2, 0.5% triton X-100, and 1% protease inhibitor cocktail (Sigma-Aldrich®). Total protein content was estimated using the Bradford protein assay, (Bio-Rad, Hercules, CA, USA) and stored at −80 °C.

Proteins were separated using SDS-PAGE 10% gels loaded with 80 µg of protein per sample, transferred to PVDF (Polyvinylidene Difluoride) membrane and blocked for 1 h at room temperature with 3% BSA (Bovine Serum Albumin, Sigma-Aldrich®) in TBS1x (Tris-Buffered Saline) for phosphorylated proteins or 3% nonfat dry milk in TBS1x for non-phosphorylated proteins. Subsequently, the membranes were incubated for 12 h at 4 °C with the primary antibodies (Table 3) diluted in blocking solution.

After 12 h of incubation, membranes were washed three times for 5 min with Tris-Buffered Saline and 0.1% Tween® (TBSt) and incubated with the secondary antibody (anti-rabbit IgG- Cell signaling® and anti-mouse IgG - Sigma-Aldrich®) diluted 1:5000 in blocking solution for 1 h at room temperature. Membranes were subsequently washed with TBSt, revealed by ECL ClarityTM substrate (Bio-Rad, Hercules, CA), the images were generated using a c-Digit® Blot device (Licor Biosciences, Nebraska, USA), and bands were quantified by densitometry using the software Image Studio Digits Lite Version 5.2 (Licor Biosciences). Due to the number of animals in the study, all samples could not be included on the same gel. Each SDS-PAGE gel, therefore, contained protein extracted from all the treatments, as well as an internal loading control sample used for signal normalization. For the internal control, there was use of two reference samples from the same tissue and experiment loaded on each gel. The internal control which had a greater band intensity (expressed by optic densitometry units) was used to normalize the remaining samples as described by Cruzen et al. (2014).

**Table 3**
List of antibodies used in Western blotting analysis.

| Antibody | Source | Dilution | Manufacturer | Catalog Number |
|---|---|---|---|---|
| INSR | Rabbit polyclonal IgG | 1:500 | Boster Bio-engineering® | PA1620 |
| IRS-1 | Rabbit polyclonal IgG | 1:500 | Boster Bio-engineering® | PA2269 |
| GLUT4 | Rabbit polyclonal IgG | 1:500 | Boster Bio-engineering® | PB9109 |
| AMPK | Rabbit monoclonal IgG | 1:500 | Cell signaling Technology® | 2603 |
| p-AMPK | Rabbit monoclonal IgG | 1:500 | Cell signaling Technology® | 2535 |
| p-mTOR | Mouse monoclonal IgG | 1:500 | Santa Cruz Biotechnology® | 293133 |
| PDGFRα | Rabbit monoclonal IgG | 1:500 | Boster Bio-engineering® | M00366 |
| PPARγ | Rabbit polyclonal IgG | 1:500 | Boster Bio-engineering® | PA1320 |
| MYO1B | Rabbit polyclonal IgG | 1:500 | Thermo Fisher Scientific® | PA522347 |

### 2.8. Statistical analysis

Data collected from does and newborn kids were analyzed using similar procedures. For both doe and kid data, a full fixed-effect model was used and specific model terms were removed from the model when the *P*-value > 0.10. The following full model was tested:

$$Y_{ijk} = \mu + D_i + T_j + (DT)_{ij} + BW_{ijk} + e_{ijk}$$

where, $Y_{ijk}$ is the observed measurement; μ is the overall mean; $D_i$ is the fixed-effect of the *i*th level of maternal dietary treatment (two levels); $T_j$ is the fixed effect of the *j*th level of twins (two levels; "yes" or "no"); $DT_{ij}$ is the interaction between *D* and *T*; $BW_{ijk}$ is the covariate of initial body weight of the kth doe or birth weight of the kth kid; and $e_{ijk}$ is the random error associated with $Y_{ijk}$, with $e_{ijk} \sim N(0, \sigma_e^2)$.

For each of the tissue variables analyzed, effects in the model, with the exception of *D* and *T*, were removed when there was a *P*-value > 0.10. Prior to the final analyses, extreme data were removed when Studentized residuals were not within ± 3 standard deviations, and normality (*P*-value > 0.05) was assessed using Shapiro-Wilk's test. As expected, the mRNA relative abundance data were not normally distributed and were transformed using the natural logarithm of the relative abundance values +1 to achieve normality (Voge et al., 2004). Least-squares means were separated using the Fisher's least significant difference test. Results were deemed significant when the *P*-value < 0.05. All analyses were performed using SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

## 3. Results

### 3.1. Maternal intake as related to feed restriction

The maternal dry matter intake (DMI), crude protein intake CPI and total digestible nutrients intake (TDNI) during the first experimental period (8–84 d of gestation) differed between treatment groups. The does which were feed restricted during this period (treatment R-M) had less intake ($P < 0.001$) than does from the M-R group. During the last experimental period (85d-parturition), with the exception of TDNI ($P = 0.249$), the does which were feed restricted during this period (treatment M-R) had less DMI and CPI ($P < 0.001$) than the does from the R-M group (Table 4).

**Table 4**
Intake (mean ± SEM) according to feed restriction of the does during different stages (first or second half) of gestation.

| Variable[a] | Treatment | | *P*-value |
|---|---|---|---|
| (g/day) | M-R[b] | R-M[c] | |
| 8 – 84d of gestation | | | |
| DMI | 851 ± 29.7 | 432 ± 34.3 | < 0.001 |
| CPI | 79.0 ± 4.03 | 41.2 ± 4.67 | < 0.001 |
| TDNI | 581 ± 22.2 | 393 ± 25.7 | < 0.001 |
| 85d – parturition | | | |
| DMI | 719 ± 32.3 | 982 ± 37.3 | < 0.001 |
| CPI | 64.0 ± 2.48 | 87.9 ± 2.86 | < 0.001 |
| TDNI | 533 ± 16.4 | 503 ± 18.9 | 0.249 |

[a] Dry matter intake (DMI), crude protein (CPI) and total digestible nutrients intake (TDNI).
[b] M–R: maintenance-restriction treatment.
[c] R-M: restriction-maintenance treatment.

**Table 5**
Least-Squares means ± standard error means for maternal performance in does fed the differing feed-restricted diets during different stages (first or second half) of gestation.

| Variable[a] | Treatment | | *P*-value |
|---|---|---|---|
| (g/day) | M-R[b] | R-M[c] | |
| 8 – 84d of gestation | | | |
| Total ADG | 95.5 ± 7.99 | −1.24 ± 9.23 | < 0.001 |
| Maternal tissues ADG | 75.0 ± 8.24 | −24.6 ± 9.25 | < 0.001 |
| Gestation ADG | 22.2 ± 2.44 | 22.2 ± 2.83 | 0.999 |
| 85d – parturition | | | |
| Total ADG | 27.3 ± 15.4 | 191 ± 17.8 | < 0.001 |
| Maternal tissues ADG | −80.5 ± 15.3 | 82.1 ± 17.8 | < 0.001 |
| Gestation ADG | 107 ± 11.4 | 109 ± 13.2 | 0.931 |
| 8d – parturition | | | |
| Total ADG | 77.0 ± 8.61 | 88.2 ± 9.94 | 0.411 |
| Maternal tissues ADG | 17.4 ± 8.48 | 24.9 ± 9.79 | 0.569 |
| Gestation ADG | 60.9 ± 6.37 | 62.2 ± 7.36 | 0.894 |

[a] Total average daily gain (ADG), maternal tissues ADG and gestation ADG of does from the first (8–84d of gestation), the last (85d to parturition) experimental periods, and during the entire experimental period (8d to parturition).
[b] M–R: maintenance-restriction treatment.
[c] R-M: restriction-maintenance treatment.

### 3.2. Maternal performance as related to feed restriction

The ADG of the does, during the first (8–84d of gestation) and last (85d-parturition) experimental periods differed ($P < 0.001$) between treatment groups. There, however, was no difference ($P = 0.41$) among treatment groups when there was calculation of maternal ADG during the entire gestation period (day 8 to parturition) (Table 5).

Similarly, the ADG of maternal tissues was different ($P < 0.001$) between treatment groups and experimental periods (Table 5). The does from the R−M treatment group, while there was feed restriction in the first experimental period, gained less weight than does from the M-R treatment group. Conversely, the doss from the M-R treatment group had less weight gain than the does from the R-M treatment group during the last experimental period. The ADG of does during the whole gestation was similar between treatment groups ($P = 0.57$).

The weight of the fetal tissues remained similar between the groups from 8–84 days of gestation ($P = 0.99$), and from 85 days of gestation until parturition ($P = 0.93$). Consequently, there were no differences of the weight of fetal tissues between treatment groups when evaluated during the entire gestation ($P = 0.89$; Table 5).

### 3.3. Birth weight and blood glucose concentrations of newborn kids

There were no differences for birth weight ($P = 0.46$), or blood glucose concentrations ($P = 0.65$) of kids born from does that were feed-restricted from 8–84 days of gestation (R-M) compared to those born from does that were feed-restricted from 85 days of gestation to parturition (M-R; Fig. 1).

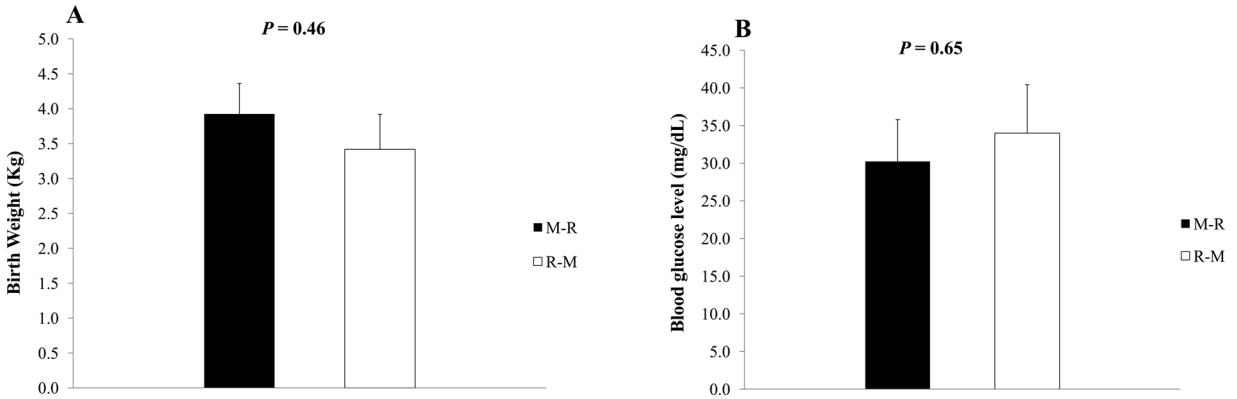



**Fig. 1.** Average birth weight (A) and blood glucose concentration (B) of dairy goat kids at the time of birth from does fed at maintenance from 8–84 days of gestation followed by feed- restriction from 85 days of gestation to parturition (M–R), and kids at the time of birth from does that were feed restricted from 8–84 days of gestation followed by feeding at maintenance from 85 days of gestation to parturition (R-M); Results are presented as Least-Squares Means ± SEM.

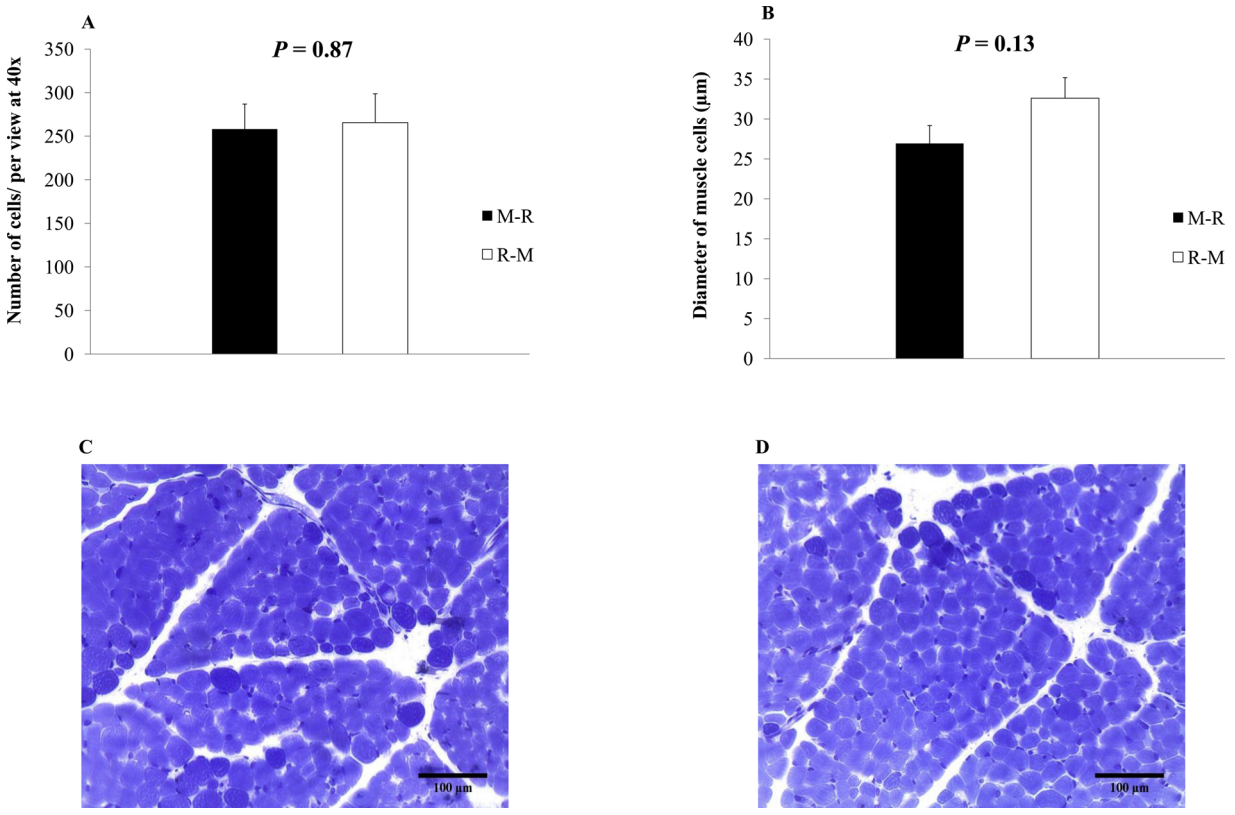



**Fig. 2.** Cell number (A) and size (B) of *Longissimus dorsi* muscle of dairy goat kids at the time of birth from does fed at maintenance from 8–84 days of gestation followed by feed- restriction from 85 days of gestation to parturition (M–R) (C), and kids at the time of birth from does that were feed restricted from 8–84 days of gestation followed by feeding at maintenance from 85 days of gestation to parturition (R-M) (D); Skeletal muscle samples were embedded in resin, 3 µm sectioned, stained with toluidine blue and visualized in 40-fold magnification; Results presented as Least-Squares Means ± SEM (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

### 3.4. Skeletal muscle cell number and size of kids at birth

There were no differences in cell number ($P = 0.87$) or cell size ($P = 0.12$) of kids born from does that were feed-restricted from 8–84 days of gestation (R-M) compared to those born from does that were feed restricted from 85 days of gestation to parturition (M–R; Fig. 2).

### 3.5. Molecular markers of cell fate in skeletal muscle of kids at time of birth

There was no difference in relative abundance of *PAX3* mRNA ($P = 0.94$), a marker of myogenic events, in skeletal muscle of kids at the time of birth between the R—M and M–R treatment groups (Table 6). For the relative abundance of *PAX7* mRNA, a marker of satellite cells, there was an interaction between number of fetuses (single or twin) and maternal nutritional treatment during gestation ($P < 0.001$; Fig. S1).

Abundance of mRNA for adipogenic markers such as *ZFP423* ($P = 0.79$), *PPARγ* ($P = 0.19$) and the inhibitor of adipogenesis *PREF-1* ($P = 0.84$) were not different between treatment groups (Table 6). There was no difference in mRNA abundance for *COL I* ($P = 0.28$; Table 6), a fibrogenic marker, neither in total collagen content ($P = 0.14$; Fig. 3) between the R—M and M–R treatment groups. Relative abundance of mRNA for *COL III* ($P = 0.08$) tended to be greater in the R—M than M–R treatment groups. Furthermore, the relatively abundance of mRNA for *PDGFRα*, a growth factor receptor and mesenchymal stem cell marker, did not differ between treatment groups ($P = 0.87$; Table 6). There were no differences in the abundance between the two groups as assessed using western-blot analysis of PDGFRα ($P = 0.82$) and the adipogenic marker PPARγ ($P = 0.86$; Fig. 4).

### 3.6. Molecular markers of energy metabolism in skeletal muscle and liver of kids at birth

There were no differences in relative abundance of mRNA between the R—M and M–R treatment groups for the markers related with energy status in the skeletal muscle, including *INSR* ($P = 0.75$), *IRS*$^{-1}$ ($P = 0.91$), and *GLUT4* ($P = 0.19$) (Table 7). The relative abundance of mRNA for *HKII* ($P = 0.03$) was greater in skeletal muscle of kids at the time of birth of the R—M than M–R treatment

**Table 6**
Least-Squares means ± standard error means for mRNA relative abundance of myogenic, adipogenic, fibrogenic and mesenchymal stem cell markers evaluated in dairy goat kid skeletal muscle when kids were from does fed diets with differing patterns of nutritional restriction.

| Variable[a] (Arbitrary units) | Treatment | | *P*-value |
|---|---|---|---|
| | M-R[b] | R-M[c] | |
| Myogenic marker | | | |
| PAX3 | 2.56 ± 0.48 | 2.50 ± 0.55 | 0.938 |
| Adipogenic markers | | | |
| Zfp423 | 1.66 ± 0.25 | 1.76 ± 0.28 | 0.791 |
| PPARγ | 1.40 ± 0.18 | 1.79 ± 0.21 | 0.191 |
| PREF-1 | 4.28 ± 0.80 | 4.03 ± 0.92 | 0.842 |
| Fibrogenic markers | | | |
| COLI | 4.19 ± 0.37 | 3.55 ± 0.43 | 0.283 |
| COLIII | 1.39 ± 0.11 | 1.72 ± 0.13 | 0.081 |
| Mesenchymal stem cell marker | | | |
| PDGFRα | 2.04 ± 0.42 | 1.94 ± 0.48 | 0.873 |

[a] PAX3: Paired box 3; Zfp423: Zinc finger protein 423; PPARγ: Peroxissome proliferator activated-receptor gamma; PREF-1: Preadipocyte factor 1; COLI: Collagen type I, alpha 1; COL III: Collagen type III, alpha 1; PDGFRα: Platelet-derived growth factor receptor A.
[b] M–R: maintenance-restriction treatment.
[c] R-M: restriction-maintenance treatment.

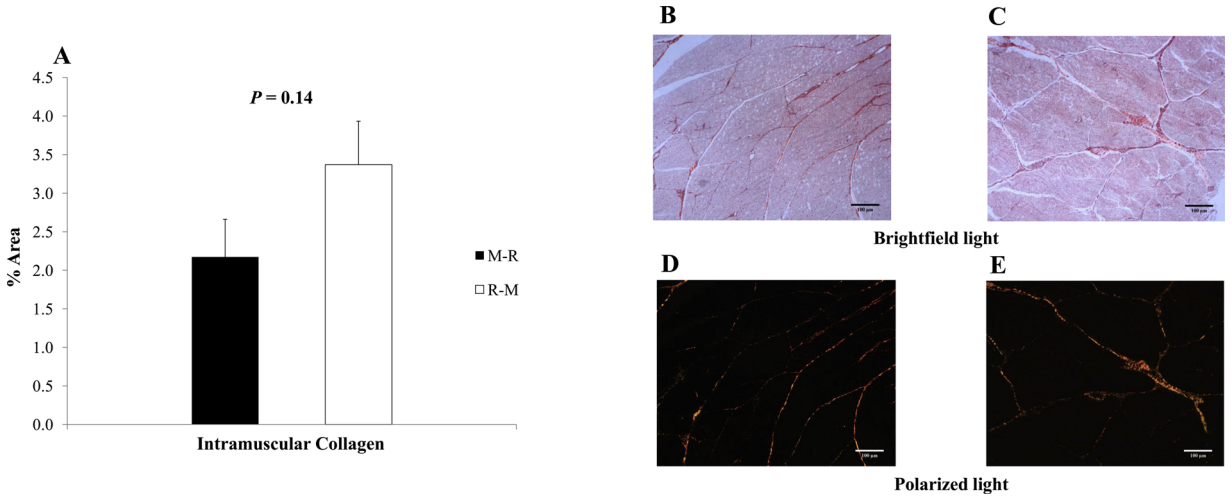
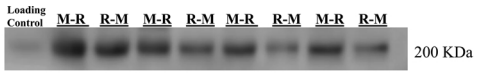


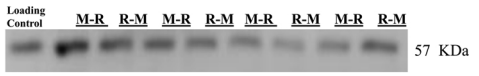

**Fig. 3.** Collagen content in skeletal muscle of dairy goat kids at the time of birth from does that were fed at maintenance from 8–84 days of gestation followed by feed- restriction from 85 days of gestation to parturition (M–R), and kids at birth from does that were feed restricted from 8–84 days of gestation followed by feeding at maintenance from 85 days of gestation to parturition (R-M) (A); Representative images of skeletal muscle stained with Sirius-red and observed using brightfield light (B and C) and polarized light (D and E) at 4-fold magnification; Results presented as Least-Squares Means ± SEM (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

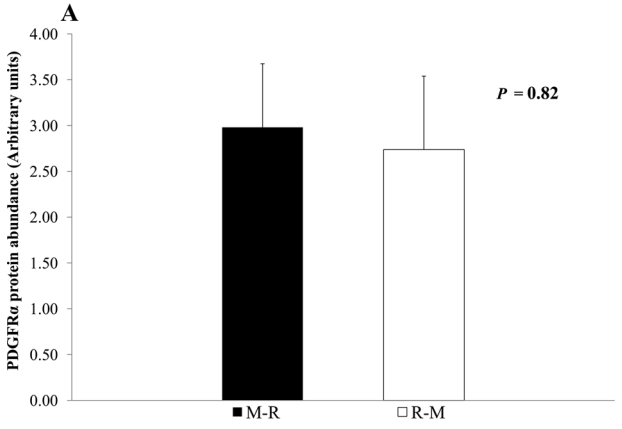
groups, while mRNA relative abundance of *PFKM* ($P = 0.15$) and *PKM* ($P = 0.63$) mRNA was not different between groups (Table 7).

There were no differences in the abundance of proteins related with energy metabolism, such as $IRS^{-1}$ ($P = 0.94$), GLUT4 ($P = 0.54$), AMPK ($P = 0.89$), the active form p-AMPK ($P = 0.99$), and p-mTOR ($P = 0.57$; Fig. 5). There, however, was an interaction for abundance of INSR between number of fetuses (single or twin) and maternal nutritional treatment during gestation ($P = 0.05$; Fig. S2). The type of skeletal muscle fiber was evaluated by assessing protein abundance for MYO1B ($P = 0.93$) which did not differ between treatment groups (Fig. 6).

With regard to energy metabolism markers in the liver, there were no differences in mRNA relative abundance for *INSR* ($P = 0.22$), *IRS*$^{-1}$ ($P = 0.80$), *HK4* ($P = 0.32$) and *PKLR* ($P = 0.28$) between the R−M and M–R treatment groups (Table 7).



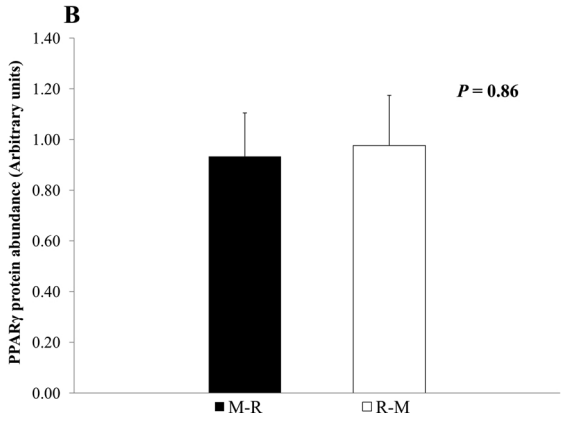




**Fig. 4.** Representative images of western-blot analysis and protein abundance for PDGFRα (A), PPARGγ (B); Protein abundance was measured in skeletal muscle of dairy goat kids at the time of birth from does fed at maintenance from 8to 84 days of gestation followed by feed- restriction from 85 days of gestation to parturition (M–R), and kids at the time of birth from does that were feed restricted from 8–84 days of gestation followed by feeding at maintenance from 85 days of gestation to parturition (R-M); Results presented as Least-Squares Means ± SEM.

**Table 7**
Least-Squares means ± standard error means for mRNA relative abundance of makers related with energy metabolism evaluated in muscle and liver of dairy goat kids from does fed diets with different patterns of feed restriction.

| Variable[a] (Arbitrary units) | Treatment | | *P*-value |
|---|---|---|---|
| | M-R[b] | R-M[c] | |
| Skeletal muscle | | | |
| INSR | 2.27 ± 0.32 | 2.43 ± 0.37 | 0.748 |
| IRS-1 | 1.63 ± 0.29 | 1.58 ± 0.34 | 0.909 |
| GLUT4 | 0.89 ± 0.06 | 1.02 ± 0.07 | 0.189 |
| HKII | 1.00 ± 0.09 | 1.31 ± 0.08 | 0.034 |
| PFKM | 0.91 ± 0.08 | 1.10 ± 0.09 | 0.146 |
| PKM | 5.41 ± 0.90 | 4.73 ± 1.04 | 0.629 |
| Liver | | | |
| INSR | 1.25 ± 0.17 | 0.92 ± 0.18 | 0.223 |
| IRS-1 | 2.28 ± 0.47 | 2.10 ± 0.51 | 0.805 |
| HK4 | 3.74 ± 0.99 | 5.30 ± 1.15 | 0.325 |
| PKLR | 3.06 ± 0.67 | 1.91 ± 0.77 | 0.282 |

[a] INRS: Insulin receptor; $IRS^{-1}$: Insulin receptor substrate 1; GLUT4: Solute carrier family 2 member 4; HKII: Hexokinase II; PFKM: Phosphofructokinase muscle; PKM: Pyruvate kinase muscle; HK4: Glucokinase (Hexokinase 4); PKLR: Pyruvate kinase liver and red blood cell.
[b] M-R: maintenance-restriction treatment.
[c] R-M: restriction-maintenance treatment.

## 4. Discussion

### *4.1. Maternal feed restriction at different stages of gestation and skeletal muscle development*

The difference in maternal total ADG in the present study, and especially in the ADG of weight of maternal tissues between periods highlights the efficacy of the maternal nutritional treatment applied in the present study. This indicates the total ADG and maternal tissue ADG at the end of the experimental period was the same between treatment groups, and only differed during different periods (feed-restriction x maintenance at 8–84 days of gestation or 85 days of gestation to parturition). Furthermore, the increase in body weight gain of the does in both treatment groups that occurred during the last period can be attributed to the greater fetal growth that occurs at this stage compared to the first half of gestation.

Nonetheless, the difference in maternal weight gain did not appear to affect fetal growth, as well as the gestation ADG and weight

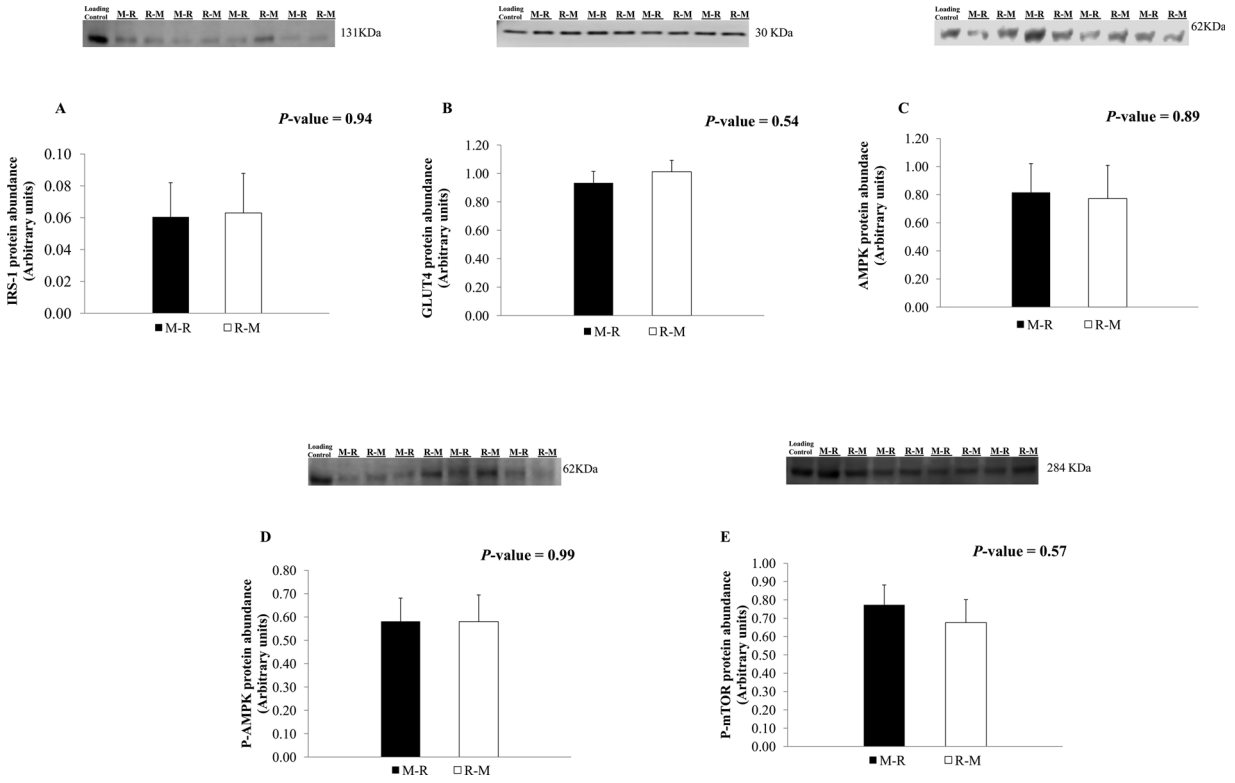



**Fig. 5.** Representative images of western blotting analysis and protein abundance for $IRS^{-1}$ (A), GLUT4 (B), AMPK (C), P-AMPK (D) and P-mTOR (E); Protein abundance was measured in skeletal muscle of dairy goat kids at the time of birth from does fed at maintenance from 8–84 days of gestation followed by feed- restriction from 85 days of gestation to parturition (M–R), and kids at the time of birth from does that were feed restricted from 8–84 days of gestation followed by feeding at maintenance from 85 days of gestation to parturition (R-M); Results presented as Least-Squares Means ± SEM.

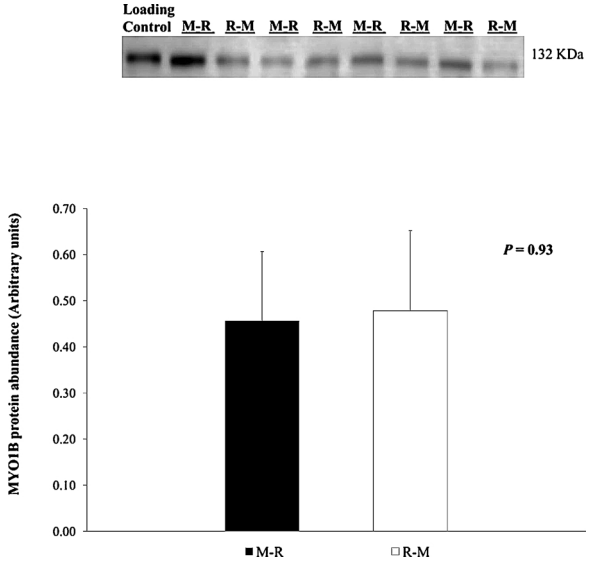



**Fig. 6.** Representative images of western blotting analysis and protein abundance for MYO1B; Protein abundance was measured in skeletal muscle of dairy goat kids at the time of birth from does that were fed at maintenance from 8to 84 days of gestation followed by feed- restriction from 85 days of gestation to parturition (M–R), and in kids at the time of birth from does that were feed restricted from 8–84 days of gestation followed by feeding at maintenance from 85 days of gestation to parturition (R-M); Results presented as Least-Squares Means ± SEM.

of kids at the time of parturition were similar, which corroborates results of other studies (Wu et al., 2006; Du et al., 2010; Underwood et al., 2010) and indicates birthweight might not be affected by maternal nutrition status.

Maternal feed restriction was associated with a decrease in muscle fiber number in the offspring when imposed before the second wave of myogenesis (approximately 105 d of gestation in ewes and 210 d in cattle; Du et al., 2010). Subsequent to this time period, there is no evidence of an increase in number of muscle fibers, thus, postnatal muscle growth is mainly due to an increase of muscle fiber size. It was hypothesized, therefore, that newborns from the R−M treatment groups would have less and larger muscle fibers than kids of the M–R treatment group. There were, however, no differences in cell number and size between treatment groups. At the day in which the treatment was changed (day 85), the secondary phase of myogenesis was still occurring and even though there was imposition of dietary restriction since day 8 of gestation, it is suggested that there was a compensation in development of tissues when the treatment was changed. This likely resulted in the similarities in muscle characteristics among kids at the time of birth of both treatment groups, in terms of number and size of muscle cells.

A genome-wide analysis indicated that overexpression of *PAX3* and *PAX7* genes resulted in an enhanced expression of genes associated with growth and proliferation and decreased expression of genes related to myogenic differentiation (Buckingham and Relaix, 2015). Considering the importance of the transcriptional factors, *PAX3* and *PAX7,* for muscle development and growth, it is essential that *Pax3/7* expressing cells remain in the fetus throughout gestation and adult life (Gonzalez et al., 2013). Based on these finding, results in the present study related to *PAX7* gene expression indicated there was an interaction between number of fetuses (single or twin) and maternal nutritional treatment. A greater expression of the *PAX7* gene in singletons from the R−M group and in twins from the M–R group may be related to the greater number of satellite cells present postnatally and further increase in number of muscle fibers that subsequently are available for new fiber proliferation. The PAX7 protein functions in recruitment of the histone methyltransferase complex (Trithorax complex) leads to chromatin modification by the methylation of histone H3 lysine 4 (H3K4), an epigenetic marker that stimulates transcriptional activation of myogenic regulatory factors (MRFs; Buckingham and Relaix, 2015; Sincennes et al., 2016). Additionally, there were changes in temporal effects of MRFs and decreased fusion index in satellite cells cultured from offspring of restricted-fed ewes (Raja et al., 2016), which may lead to impaired muscle growth postnatally.

In addition to myogenesis, there are events related with adipogenesis and fibrogenesis that can be affected by maternal nutrition (Huang et al., 2012; Duarte et al., 2014; Marquez et al., 2017; Paradis et al., 2017). The formation of muscle cells is less as gestation progress concomitantly with the increase of adipogenesis and fibrogenesis (Du et al., 2010). The imposition of intrauterine growth restriction induces enhancement of adipogenesis postnatally and leads to obesity (Ross and Desai, 2013). The mechanisms involved in increasing the rate of adipogenesis resulted in programmed appetite/satiety and function (lipogenesis; García et al., 2010; Fukami et al., 2012; Ross and Desai, 2013). Furthermore, results of previous studies indicate mesenchymal progenitor cells are committed to adipogenic and fibrogenic lineages (Joe et al., 2010; Uezumi et al., 2010), which directly contribute to intramuscular fat and connective tissue development (Uezumi et al., 2010, 2011). The marker gene, *PDGFRα*, is specifically expressed in skeletal muscle and represent a cell population distinct from satellite cells (Uezumi et al., 2014).

In the present study, there was assessment of PDGFRα content as a marker of mesenchymal progenitor cells, and the results indicate that imposition of maternal feed restriction at both developmental stages where evaluations occurred did not affect the number of these cells in the kids at the time of birth. Concomitantly, the mRNA relative abundances of the marker gene mRNAs related with adipogenesis were also not altered by treatments. Similarly, collagen content and the mRNA relative abundances of *COL I* were not different between treatment groups, indicating that the maternal feed restriction in both periods when evaluations occurred did not affect fibrogenesis. The expression of the fibrogenic marker gene, *COL III*, tended to be less at the time of birth in kids of does from the M–R treatment group. These data indicate that maternal feed restriction in the second half of gestation does not affect the adipose tissue formation/fat deposition and may contribute to a decrease in formation of connective tissue by decreasing *COL III* gene expression.

### *4.2. Maternal feed restriction at different stages of gestation and skeletal muscle energy metabolism*

As a result of interpretation of results in several studies, there appears to be an association between intrauterine growth restrictions with alterations in energy metabolism, including insulin pathways (George et al., 2012; He et al., 2013; Aragão et al., 2014). Compared with vital organs, there is a lesser energy partitioning to skeletal muscle in the developing fetus. Maternal feed restriction may, therefore, lead to an adaptive response in energy metabolism in offspring at the time of birth to maintain glucose supply to vital organs (Thorn et al., 2013). In response to glucose, insulin is secreted and its pathways are highly regulated and sensitive to changes in oxygen, nutrient status, and cell stress as a result of signals from several major energy and stress sensors including AMP-activated protein kinase (AMPK; Thorn et al., 2009). The AMPK functions as an important cellular energy sensor and is activated by phosphorylation and increase in the intracellular AMP:ATP ratio. In skeletal muscle, its activation stimulates glucose uptake by enhancing the glucose transporter GLUT4 translocation, fatty acids oxidation, mitochondrial biogenesis, inhibition of glycogen synthesis, and protein synthesis because of inhibition of mTOR (Wang et al., 2012; Coughlan et al., 2014). Also, when activated, AMPK regulates glycolysis pathways by phosphorylation of phosphofructokinase 2 (PFK2) (Sanchez et al., 2012) to promote breakdown of glucose to extract energy for cellular metabolism. Hence, maternal feed restriction promotes an increase in the activated AMPK. No differences, however, were observed in protein abundance of p-AMPK or p-mTOR. Results from the present study indicate that even with feed restriction of the dams there were sources of ATP precursors, such as glucose for fetal growth.

Decreased muscle glucose uptake is related to decreased expression of the *HKII* gene (Vestergaard et al., 1995; Wang et al., 2016). Even when there was not a differential in GLUT4 abundance in the present study, the kids at the time of birth from does of the M–R group had a lesser abundance of *HKII* mRNA. Interestingly, abundance of mRNA for the enzymes that catalyze glycolysis downstream

G6-P was not affected by maternal feed treatment during gestation. Although enzymes associated with glycogen synthesis were not evaluated in the present study, findings indicate maternal feed intake restriction during the second half of gestation impaired the storage of glucose as glycogen in skeletal muscle of the kids.

When there was a lack of energy substrates due to imposition of intrauterine growth restriction on fetuses, there were changes in tissue fiber composition which was closely related to oxidative capacity of muscle and insulin sensitivity (He et al., 2001; Oberbach et al., 2006; Coen et al., 2010; Stuart et al., 2013). Results of previous studies indicate there is a positive correlation between slow twitch (type I) muscle fibers and insulin sensitivity. Results involving changes in fiber composition have been variable among studies. In response to maternal undernutrition during early to mid-gestation, there was an increase (Zhu et al., 2006; Daniel et al., 2007) or decrease in the number of fast twitch (type II) muscle fibers postnatally. While with the imposing of undernutrition during late gestation, there was a decrease in slow twitch (type I) muscle fibers in the fetuses (Costello et al., 2008). Furthermore, in lambs from ewes in which there was restricted feed intake during the intrauterine period, there was a greater abundance of type IIa fibers at birth. When lambs were 3 months of age, however, there were no differences in responses among treatment groups in skeletal muscle fiber types, indicating that feeding of an adequate dietary nutrient content postnatally resulted in fiber type switching (Reed et al., 2014). Thus, in the present study maternal feed restriction during the first half of gestation did not affect muscle fiber composition in kids at the time of birth or there was a compensation which allowed for restoration of fiber composition when the does were fed diets that favored fetal growth after a period of feed restriction.

## 5. Conclusion

Even though there was a small animal sample size in the present study, results indicate maternal feed restriction of dairy goat does at early or late gestation does not differentially affect relative abundance of mRNA for myogenic, adipogenic and fibrogenic markers or mesenchymal stem cell population in skeletal muscle of kids at the time of birth. Energy metabolism, however, may be impaired by reducing the expression of the *HKII* gene in skeletal muscle of dairy goat kids at the time of birth. Collectively, the results of the present study indicate maternal realimentation of does during the second half gestation that were feed-restricted during the first half of gestation results in negation of any impairment of skeletal muscle development in dairy goat kids.

## Declaration of interest

The authors have nothing to disclose.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2019.05.006.

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