R. Bras. Zootec., 52:e20230051, 2023
https://doi.org/10.37496/rbz5220230051

Ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Development of the gastrointestinal tract of newborn goats under maternal feed restriction at different stages of gestation

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Received: March 29, 2023
Accepted: October 3, 2023

How to cite: Santos, L. R.; Costa, T. C.; Souza, R. O.; Gionbelli, T. R. S.; Oliveira Junior, I. M.; Ramírez-Zamudio, G. D.; Nascimento, K. B.; Duarte, M. S. and Gionbelli, M. P. 2023. Development of the gastrointestinal tract of newborn goats under maternal feed restriction at different stages of gestation. Revista Brasileira de Zootecnia 52:e20230051. https://doi.org/10.37496/rbz5220230051

ABSTRACT - This study evaluated how nutritional insults applied at different stages of intrauterine development affect the growth and development of total tract organs and the mRNA expression of genes that encode growth factors, tight junction proteins, digestive enzymes, and glucose transporters in the small intestine of newborn goats. Fourteen nulliparous dairy goats were assigned to one of two nutritional plans over gestation: maintenance-restriction (M-R, n = 8) – 100% of maintenance requirement from d 8-84 of gestation followed by feeding at 50% maintenance requirement from d 85 of gestation to parturition; or restriction-maintenance (R-M, n = 6) – 50% of maintenance requirement from d 8-84 of gestation followed by feeding at 100% maintenance requirement from d 85 of gestation to parturition. Fisher's least significant difference test was performed using SAS 9.4, considering a full fixed-effect model. When expressed per kg of body weight, the weights of the small intestine and total intestine were 18.7 and 18.1% lower, respectively, for R-M offspring. The lengths of the small intestine and the total intestine of the R-M newborns were 19.1 and 9.5% shorter, respectively. This group also had a lower villus height:intestinal crypt depth ratio. The R-M offspring tended to have lower Solute carrier family 5 member 1 (*SLC5A1*), Solute carrier family 2 member 2 (*SLC2A2*), and Occludin (*OCLN*) mRNA expressions. Therefore, feed restriction in the first half of gestation is detrimental to the second half of gestation, reducing the offspring's intestinal absorptive area but enhancing their intestinal permeability.

Keywords: fetal programming, intestine, nutrients absorption, organogenesis, small ruminants

1. Introduction

The attendance below the maternal requirements commonly verified in the dry season promoted by the challenged nutritional scenario alter maternal metabolism and body condition status (Barcelos et al., 2022) and cause long-term consequences on the offspring's growth trajectory, including the fetal organogenesis (Vonnahme, 2007; Duarte et al., 2013; Gionbelli et al., 2017), skeletal muscle development (Costa et al., 2021a; Costa et al., 2021b; Santos et al., 2022), and offspring's metabolism (Zhou et al., 2019). The nutrients supply above the maternal requirements reduces the weight of the pancreas, stomach, liver, spleen, kidneys, lungs, and other fetal organs (Zhang et al., 2016). Other studies demonstrated that maternal undernutrition during gestation leads to compromised pancreatic enzymatic capacity (Keomanivong et al., 2016), in addition to promoting fibrosis, oxidative stress, and difunction (Zi et al., 2023).

Among the effects of the maternal nutritional plan over pregnancy on offspring organs development, the effects promoted in the intestine are particularly important since the growth, development, and vascularization of the small intestine are essential processes that ensure immunological protection, nutrient metabolism, neonatal survival, and postnatal growth (Trahair and Sangild, 2002). Studies with beef cattle demonstrated that maternal nutritional restriction or the lack of supplementation during critical periods of gestation can increase the surface of the small intestine and, consequently, the absorptive capacity of the offspring (Duarte et al., 2013; Rodrigues et al., 2020). Consistently, Hammer et al. (2011) reported that lambs from ewes subjected to nutritional restriction had better immunoglobulin absorption efficiency during the first hours after birth, while Meyer et al. (2010) demonstrated that intestines of fetuses of restricted dams presented increased proliferation and vascularization. Available evidence demonstrated greater nutrient digestibility in the feedlot phase for male offspring from dams fed low protein levels during mid-gestation (Nascimento et al., 2022) and that moderate protein restriction during mid and late gestation resulted in greater gut expression of genes involved with glucose and fatty acids metabolism (Cruz et al., 2019).

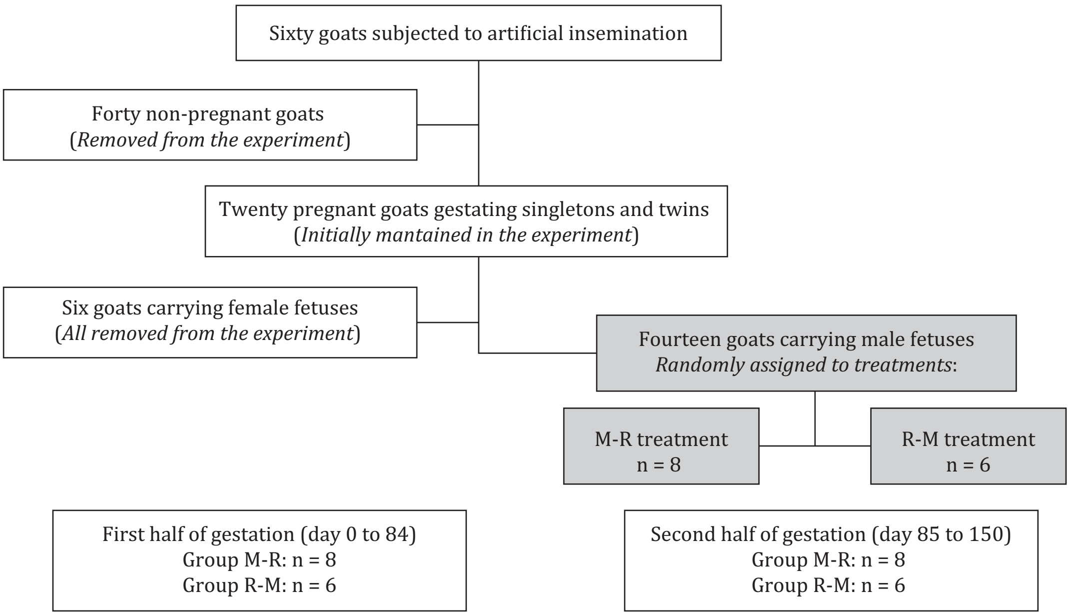
Therefore, it is unquestionable that maternal nutritional restriction affects the development and functioning of the gastrointestinal tract of the offspring in the short and long term. However, little is known about how nutritional insults applied at different stages of pregnancy affect these tissues. Therefore, the objective of this study was to understand in which period of pregnancy (first or second half of pregnancy) the nutritional insult effects may be more deleterious to the small intestine morphology and function of newborns goats.

2. Material and Methods

This study was performed in Viçosa, Minas Gerais, Brazil (Latitude: 20°45'17" South, Longitude: 42°52'57"). Research on animals was conducted according to the institutional committee on animal use (protocol no. 09/2017). Additional experimental details have been previously reported in our complementary study (Costa et al., 2019).

2.1. Animals and treatments

Fourteen nulliparous Saanen dairy goats, with 50±13 kg and 19±7 months, were subjected to an estrus synchronization protocol and artificially inseminated using semen from a single male. All goats were dewormed before the estrous synchronization protocol, specifically during the rainy period (January in Brazil). After the pregnancy confirmation (30 days after artificial insemination), all goats were evaluated through clinical observation using the FAMACHA method to detect anemia. During the experimental period, there were no signs of worms in pregnant goats. After insemination, dairy goats were allocated in individual pens [3 m^2 with concrete floors, all of which were roof-covered and equipped with individual drinkers and feeders] and subjected to an adaptation period of 7 d receiving the experimental diet and water *ad libitum*. Eight days post-conception, the dairy goats were randomly assigned to two groups: maintenance-restriction (M-R, n = 8) or restriction-maintenance (R-M, n = 6) treatments in the first or in the second half of gestation, respectively. The M-R group was individually fed at the maintenance level 100% of total digestible nutrients (TDN) and crude protein (CP) requirements according to NRC (2007) until the 84th day of gestation. From the 85th day of gestation until parturition (term ~ 150 d), they were fed 50% of TDN and CP. The R-M group was individually fed 50% of TDN and CP until the 84th d of gestation and received 100% of TDN and CP from the 85th day of gestation to the parturition date. Therefore, the treatments consisted of the same diet for all animals, with differences only in feeding regime and gestational stages. The design of the application of treatments to goats during pregnancy is shown in Figure 1.



Group M-R (feeding regimen 1): dairy goats fed at 100% of the maintenance requirement from d 8-84 of gestation, followed by feeding at 50% maintenance requirement from d 85 of gestation to parturition; group R-M (feeding regimen 2): dairy goats fed at 50% of their maintenance requirement from d 8-84 of pregnancy, followed by feeding at 100% of their maintenance requirement from d 85 of gestation to parturition.

Figure 1 - Schematic diagram of the animals used in this trial and of experimental design.

2.2. Experimental diets

The experimental diets were formulated to achieve the expected nutritional requirements of dairy goats (NRC, 2007). The diet offered during the experimental period was composed of a 72:28 roughage:concentrate ratio. The forage source used was corn silage [dry matter (DM) = 25.7%; CP = 7.12%; ether extract (EE) = 2.4%; and neutral detergent fiber corrected for ash and protein (NDFap) = 54.2%] (Table 1). The concentrate (DM = 84.5%; CP = 14.5%; EE = 0.7%; and NDFap = 14.7%) was prepared using 96 g/kg DM of soybean meal, 165 g/kg DM of ground corn, and 16 g/kg DM of mineral (Table 1).

The experimental diet was weighed and provided once daily (07:00 h). Dry matter intake (DMI) and daily supply of the diet were adjusted weekly based on the body weight and week of gestation of the dams. To make these adjustments, the dairy goats were weighed every seven days before the morning feeding. The roughage and concentrate were sampled weekly and stored at −20 °C for further analysis.

Table 1 - Feeds and chemical composition of the experimental diet

Diet component	Total
	Corn silage + concentrate[1]
Dry matter (DM, %)	42.0
Organic matter (% of DM)	95.0
Crude protein (% of DM)	9.16
Ether extract (% of DM)	1.89
Neutral detergent fiber corrected for ash and protein (% of DM)	43.3
Non-fiber carbohydrates (% of DM)	40.7
Total net energy (Mcal/kg of DM/day)[2]	1.18±0.47

[1] Considering the ratio of 72:28.
[2] Estimated through NE = ME * 0.644, in which NE = net energy and ME = metabolizable energy.

Subsequently, samples were dried in an oven (55 °C per 72 h), grounded (Wiley mill; A. H. Thomas, Philadelphia, PA) in a 1-mm sieve, and chemically analyzed following the Brazilian National Institute of Science and Technology in Animal Science guidelines (Detmann et al., 2012).

Total net energy was estimated by converting the intake of TDN, reported by Costa et al. (2019), into metabolizable energy (ME), considering that 1 kg is equivalent to 4.409 Mcal digestible energy (DE), and considering the efficiency of conversion of ME into DE of 82% (NRC, 2001). Moreover, the efficiency of conversion of ME into NE was considered 64% according to the Small Ruminant Nutrition System (SRNS) (Tedeschi et al., 2010).

2.3. Tissue sampling

After birth, male newborn goats were immediately isolated from their dams and stunned using a non-penetrating captive bolt pistol, followed by exsanguination. In the case of newborn twins (R-M, n = 4; M-R, n = 5), we chose to euthanize the heaviest animal. The liver, kidneys, and mesentery were removed and weighed. The stomach complex was isolated and stratified into reticulum-rumen omasum and abomasum, and each component was emptied and weighed. The large and small intestines were isolated, cleaned with a phosphate buffer saline (PBS; NaCl 137 mM, KC1 2.7 mM, Na_2HPO_4 10 mM, KH_2PO_4 1.8 mM, pH 5.6) solution, weighed, and the length was recorded separately, similarly to that described by Duarte et al. (2013) and Gionbelli et al. (2017). Briefly, the duodenum (segment from pylorus up to a point adjacent to the gastrosplenic vein entrance) and the jejunum were identified as the segment formed from the duodenum caudal part up to jejunum and ileum junction (Soto-Navarro et al., 2004). A cut of 20 cm representing the middle portion of jejunum was performed, and 1 cm of the ends were removed. Subsequently, one sample (3 cm) was collected from jejunum and stored in 10% (weight/volume) formalin in phosphate buffer (pH 7.4) for histological analysis, while two samples (3 cm each) were frozen and stored in a freezer at −80 °C for further RNA extraction.

2.4. Morphology of intestinal villi

Samples of jejunum, about 3 cm long, were collected in the medial portion of the intestinal section and then fixed in fresh 10% (weight/volume) formalin in phosphate buffer (pH 7.4), dehydrated in crescent ethanol series, and embedded in the resin [HistoResin Mounting Kit (Leica, Solmos, Hessen, Germany)]. Sections of 3 µm were obtained using a rotary microtome (RM 2265, Leica Biosystems, Nussloch, Germany). One in 10 serially obtained sections was stained with toluidine blue for 10 s, washed with running water, and mounted with DPX (Sigma-Aldrich). Ten photomicrographs were obtained by photomicroscope Olympus AX70 coupled with an AxioCam HRc-Zeiss camera at a magnification of 20X, to verify the villi height and crypt depth using the software ImageJ (National Institute of Health, Baltimore, MD, USA). The crypt depth and villus height were measured as the mean distance from the crypt base to the crypt-villus junction and the villus base to the villus tip, respectively. The height of the villi and the measurements of the depth of the crypt were taken from an average of 10 well-oriented crypt-villi units.

2.5. Quantitative gene expression analysis

Total RNA was isolated from 0.03 g of each pulverized sample of the jejunum of newborn goats using the SV Total RNA Isolation System kit (Promega Corporation, Madison, WI, USA), following the manufacturer's usage protocol. Total RNA concentration was quantified using the NanoVue spectrophotometer (GE Healthcare Life Sciences Inc.), and integrity was checked on 1% agarose gel. Samples were reverse transcribed into cDNA using the GoScript Reverse Transcription System Kit (Promega Corporation, Madison, WI, USA). Specific primers (Table 2) for fragment amplification of target genes and endogenous genes in the intestinal sample were synthesized and analyzed through the online programs OligoPerfect™ Designer and OligoAnalyzer 3.1, with sequences obtained from GenBank.

In this research, *β-actin* was used as the reference gene, as recommended by Zhang et al. (2018). For quantitative gene expression analysis by RT-qPCR technique, the Mastercycler® and realplex model (Eppendorf), with the SYBR Green detection system (Applied Biosystems, Foster City, CA, USA) and cDNA obtained, were used. The thermal reaction conditions comprised: 2 min at 50 °C, 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, and 15 s at 95 °C. The relative expression levels were calculated according to the method described by Pfaffl (2001).

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

Gene	Abbreviation	NCBI access code	Primer	R^2	Efficiency
Glucagon like peptide 2 receptor	*GLP-2R*	XM_005694224.3	F: CGCTGGAAAACTCCACAGAT R: GGCGTCCAACTTTTTGTTTG	0.970	1.121
Occludin	*OCLN*	NM_001082433.2	F: AGCTGCCATTGACTTCACCT R: CCTTTTTGAAAGCGTCTCCA	0.978	0.917
Solute carrier family 5 member 1	*SLC5A1*	HM060774.1	F: CGTCATCTACTTCGTGGTGGT R: GAAGAAGCCTCCAACAGTCC	0.998	0.980
Solute carrier family 2 member 2	*SLC2A2*	XM_005675321.3	F: GCAGAGTTCCGAAAGAAGAGG R: CAAAAAGCAGGTTATCTCTACATGG	0.996	1.016
Maltase-glucoamylase	*MGAM*	XM_018046851.1	F: ATCACAAGATCCTGGGACGA R: TCCGTTCCGAGTCATTTACC	0.996	1.005
Lactase	*LCT*	XM_018062350.1	F: CTCCAGAACTGCCTCTCCAC R: AAACCCAATGACGAGCACTT	0.991	1.094
Beta-actin	*ACTB*	JX046106.1	F: GTCCACCTTCCAGCAGATGT R: AGTCCGCCTAGAAGCATTTG	0.982	1.031

2.6. Statistical analysis

Data collected from dams and newborn goats were analyzed in a similar fashion. For both cases, 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:

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

in which 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 (2 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 k-th dam or birth weight of the k-th 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 variables analyzed, the effects in the model (except for D and T), were removed when P-value > 0.10. Outliers were removed when Studentized residuals were not within ± 3 standard deviations, and normality by the Shapiro–Wilk's test (P-value > 0.05). The gene expression data, which did not follow a normal distribution, were normalized using the RANK procedure of SAS (Statistical Analysis System, version 9.4). Least squares means were compared using the Fisher's test of significance. Results were deemed significant when P-value ≤ 0.05 and tendency when 0.05 < P-value ≤ 0.10. Lastly, data were presented as the least squares mean ± standard error of the mean (SEM).

3. Results

3.1. Maternal performance and intake and offspring performance and glucose levels

The effects of maternal nutrition plan over gestation on the maternal average daily gain (ADG), ADG of maternal tissues (i.e., tissue accretion related to maternal tissues discounting all tissues increase due to the pregnancy), maternal DM and nutrients intake, as well as on the offspring birth body weight and their blood parameters, were previously reported in our complementary study (Costa et al., 2019). Briefly, the findings of our complementary study showed that the maternal DM intake from 8 to 85 d, and from 85 d to parturition were ~98% and ~36% greater (P≤0.001) for M-R and R-M groups of pregnant dairy goats, respectively. Conversely, the ADG of dairy goats during the first and second half of gestation were lower (P≤0.001) for R-M and M-R treatments, respectively. There was also lower maternal tissue ADG at early (P≤0.001) and late gestation (P≤0.001), for the R-M and M-R treatments, respectively. The birth body weight was similar (P = 0.46) between R-M and M-R newborns, and so was the glucose levels (P = 0.65).

3.2. Morphometric and morphological measurements of the gastrointestinal tract of offspring

When expressed as kg body weight, there was a trend toward the lower weight of the reticulum-rumen and omasum (P = 0.057) in the R-M group compared with the M-R group (Table 3). The small intestine (P = 0.055) and total intestine (P = 0.095) tended to be lighter in newborns of the R-M treatment, and these weight differences were more pronounced (P = 0.038) when expressed as kg of body weight (Table 3). The small intestine and total intestine lengths were also higher (P≤0.04) in the M-R group. On the other hand, the weight:length ratio of the small intestine (P = 0.681) and total intestine (P = 0.464) of the offspring subjected to the different pre-natal nutritional plan were similar.

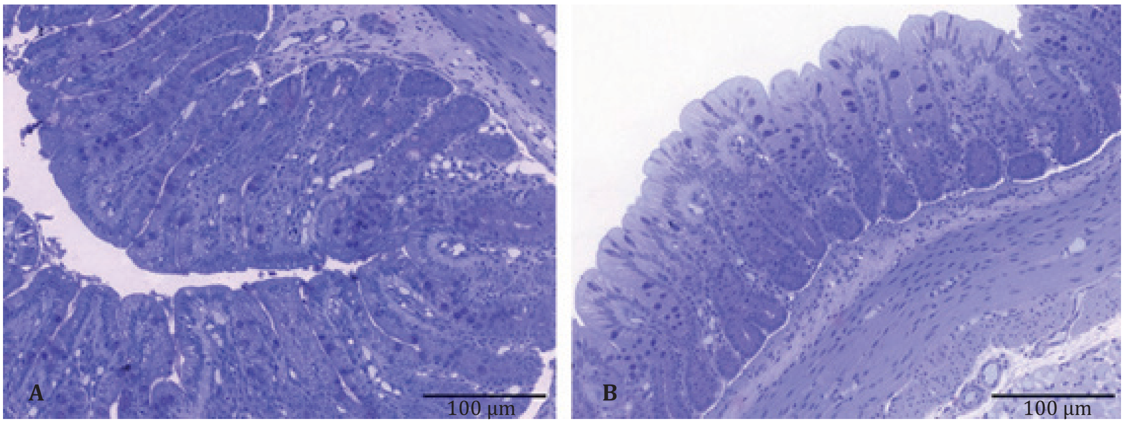
Table 3 - Effects of maternal feed restriction at different gestational moments on gastrointestinal tract measurements, organ weights, and jejunum morphology of offspring (least squares means ± SEM)

Item	Feeding regimen[1]		P-value	
	M-R	R-M	Feeding	Fetuses
	Gastrointestinal tract measurement			
Reticulum-rumen omasum (g)	18.9±0.46	18.8±0.53	0.893	0.565
Reticulum-rumen omasum (g/kg BW)	5.40±0.08	5.16±0.10	0.057	0.462
Abomasum (g)	25.0±1.25	25.3±1.44	0.862	0.465
Abomasum (g/kg BW)	6.70±0.34	6.69±0.39	0.968	0.321
Small intestine (g)	9.00±0.48	7.42±0.55	0.055	0.215
Small intestine (g/kg BW)	2.46±0.13	2.00±0.15	0.038	0.106
Small intestine (cm)	93.30±5.14	75.5±5.94	0.043	0.431
Small intestine (g/cm)	0.01±0.004	0.09±0.005	0.681	0.575
Large intestine (g)	8.57± 0.68	7.45±0.79	0.312	0.761
Large intestine (g/kg BW)	2.26±0.16	1.96±0.18	0.233	0.729
Large intestine (cm)	64.5±4.76	65.5±5.51	0.896	0.366
Large intestine (g/cm)	0.13±0.01	0.111±0.01	0.137	0.230
Total intestine (g)	17.60±0.96	14.9±1.11	0.095	0.673
Total intestine (g/kg BW)	4.71±0.215	3.95±0.248	0.038	0.573
Total intestine (cm)	157.0±3.69	142.0±4.28	0.026	0.496
Total intestine (g/cm)	0.11±0.004	0.10±0.005	0.464	0.401
	Organ weight			
Mesentery (g)	136.0±8.53	141.0±9.88	0.742	0.744
Mesentery (g/kg BW)	36.3±2.32	36.5±2.68	0.964	0.947
Kidneys (g)	18.5±0.91	18.5±1.06	0.974	0.773
Kidneys (g/kg BW)	5.02±0.21	5.06±0.24	0.896	0.859
Liver (g)	84.7±7.11	85.0±8.23	0.974	0.636
Liver (g/kg BW)	22.5±1.44	22.8±1.66	0.898	0.493
	Jejunum morphology			
Villi height (µm)	73.5±12.4	57.0±13.7	0.406	0.903
Crypt depth (µm)	98.1±9.99	130±11.6	0.081	0.128
Villus height:crypt depth ratio	0.70±0.06	0.45±0.07	0.016	0.167

SEM - standard error of the mean.

[1] M-R - maintenance-restriction treatment: 100% of maintenance requirement from d 8-84 of gestation followed by feeding at 50% maintenance requirement from d 85 of gestation to parturition; R-M - restriction-maintenance treatment: 50% of maintenance requirement from d 8-84 of gestation followed by feeding at 100% maintenance requirement from d 85 of gestation to parturition.

In addition, maternal feeding regimens did not affect large intestine measurements and organ weights of offspring (P>0.13). There was a trend towards greater crypt depth (P = 0.081) of newborn goats of the R-M group, and consequently, these animals had a lower villus height:crypt depth ratio (P = 0.016; Table 3). However, there was no difference in villus height (P = 0.406). Moreover, no fetus number effects were detected on gastrointestinal tract measurements (P≥0.106), organ weight (P≥0.493), or jejunum morphology (P≥0.167; Table 3 and Figure 2). No interaction between maternal feeding regimens and fetus number was detected for these outcomes (P≥0.05).



A: Image of offspring from dams of group M-R (feeding regimen 1): dams fed at 100% of maintenance requirement from d 8-84 of gestation, followed by feeding at 50% maintenance requirement from d 85 of gestation to parturition. B: Image of offspring from dams of group R-M (feeding regimen 2): dams fed at 50% of maintenance requirement from d 8-84 of gestation, followed by feeding at 100% maintenance requirement from d 85 of gestation to parturition.

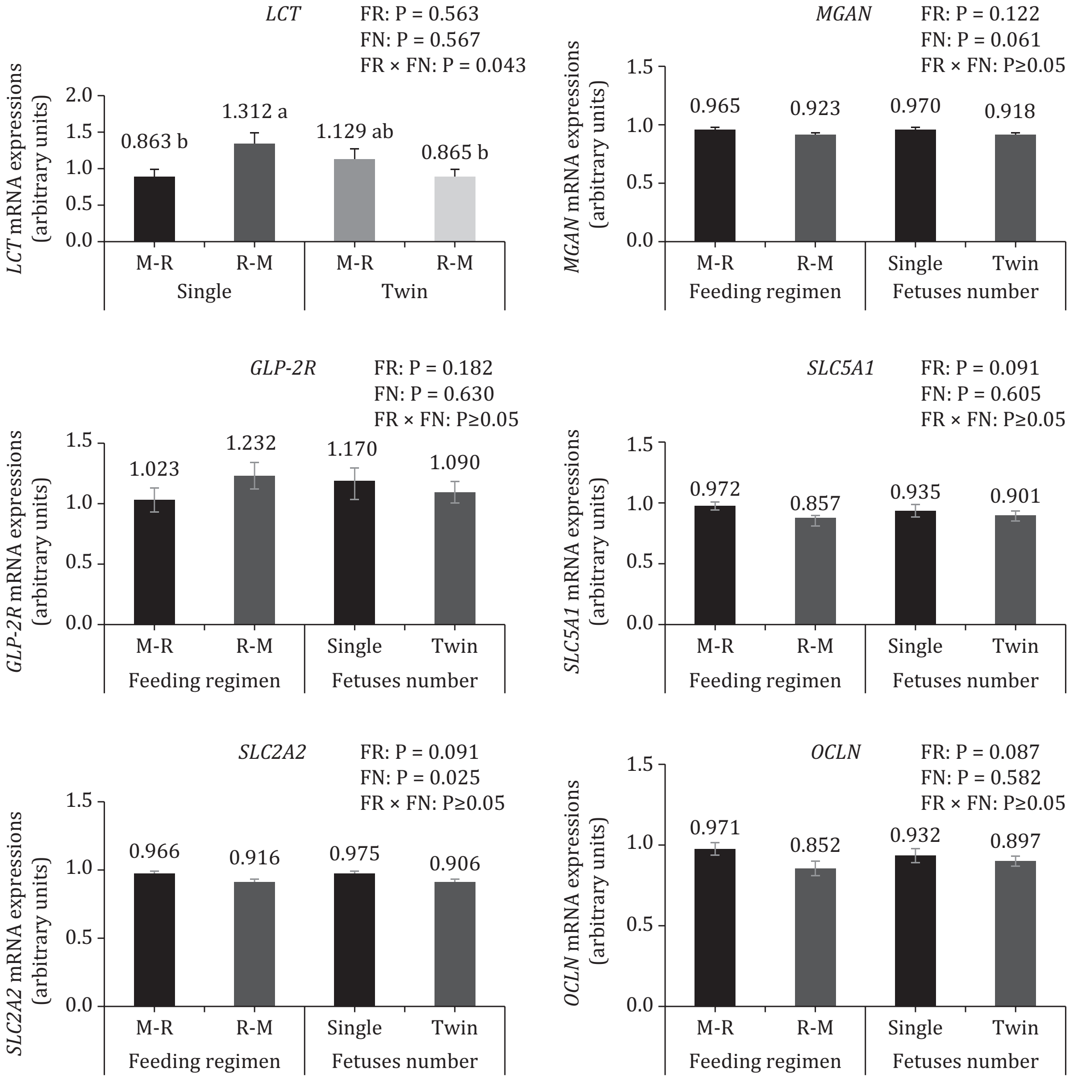
Figure 2 - Representative images of the jejunum of newborns of dairy goat kids at birth.

3.3. Gene expression in jejunum of newborn goats

No effect of maternal feed treatment was observed in the mRNA expression of the *MGAM* and *GLP-2R* in the jejunum of the offspring (P>0.120; Figure 3). However, newborns of the M-R group tended to express more mRNA of the *SLC5A1* (P = 0.091), *SLC2A2* (P = 0.091), and *OCLN* (P = 0.061). The animals born from single gestation tended to express more *MGAM* mRNA (P = 0.061) compared with animals born from twin gestation. Similarly, newborn goats from single gestation expressed more *SLC2A2* mRNA (P = 0.025). In addition, a maternal feed restriction × number of fetus interaction was observed for *LCT* mRNA expression (P = 0.043). Singletons of the R-M treatment expressed more *LCT* mRNA than the offspring of the M-R (single) and R-M (twin) groups, but no difference was observed when compared with the twins of the M-R group.

4. Discussion

The effects of maternal nutrition restriction during gestation on the small intestine of offspring are well-established in the scientific literature (Trahair et al., 1997; Meyer et al., 2010; Duarte et al., 2013; Gionbelli et al., 2017; Cruz et al., 2019; Rodrigues et al., 2020). However, to date, little is known about which periods of fetal development a nutritional restriction may be most deleterious to the development and function of the fetal gastrointestinal tract. Therefore, the experimental design proposed in this study aimed to explore this knowledge gap.



The mRNA relative abundance was measured in the jejunum of dairy goat offspring at birth.
M-R - offspring from dams fed 100% of their maintenance requirement from d 8-84 of gestation, followed by feeding at 50% maintenance requirement from d 85 of gestation to parturition; R-M - offspring from dams fed 50% of their maintenance requirement from d 8-84 of gestation, followed by feeding at 100% maintenance requirement from d 85 of gestation to parturition.
FR - feeding regimen; FN - fetuses number.
Results are presented as least squares means ± SEM.

Figure 3 - Representative images of mRNA relative abundance for digestive enzymes (LCT; MGAM), growth factors (GLP-2R), glucose transporters (SLC5A1; SLC2A2), and tight junction proteins (OCLN).

The efficacy of maternal nutritional treatment applied at each stage of gestation was confirmed by maternal ADG and maternal tissue changes (Costa et al., 2019). Besides the lack of difference in birth weight (Costa et al., 2019), the skeletal muscle transcriptome (Costa et al., 2021c) and proteome (Costa et al., 2022) profile were altered due to the maternal feed restriction, indicating that the overall offspring development and metabolism were somehow altered to adapt to maternal nutritional status.

Consistently, in the current study, the gastrointestinal tract development and function were affected by the intrauterine development window in which the nutritional restriction was applied. It is established that in the absence of adequate nutrition, the fetus sacrifices tissues, such as the intestine and muscle, for the prioritization of vital organs, such as the brain (Desai et al., 2005; Fall, 2009). Available evidence indicates that the brain mass of fetuses is preserved in situations of high nutritional stress at the cost of less important organs, such as the visceral organs (McMillen et al., 2001). Such evidence is inserted in the "thrifty phenotype theory" (Hales and Barker, 2001), which proposes that nutrient scarcity during specific windows of development leads to a nutrient reallocation to favor critical organs for immediate survival at the expense of other organs secondarily necessary. Therefore, visceral organs seem to be particularly sensitive to nutrient restriction (Reed et al., 2007).

Moreover, organogenesis is concentrated during early to mid-gestation in ruminant animals (Meyer et al., 2010). The total gastrointestinal tract, reticulum, rumen, omasum, and liver grow more at the beginning than at the end of gestation, since bovine fetuses present a greater mass of these organs per unit of body weight at 125 d of gestation (Meyer et al., 2010). The results observed in the present study for reticulum-rumen omasum, small intestine, and total intestine confirm that the mass proportional to the fetus or the absolute mass of these compartments is more significantly affected by a nutritional insult over early and mid-gestation. During the pre-weaning stages, the rumen is not active in digesting fibers and, therefore, the utilization of the chemical components produced in the fermentation process will be assimilated as the animal ages (Zhou et al., 2022). Considering that maternal feed restriction during the first half of gestation tended to impair the growth of reticulum-rumen omasum as a function of body weight, it is likely that the resulting offspring may be less efficient in terms of nutrient digestion and absorption. However, whether the digestible capacity could be altered in these offspring, through strategical starter diets (Hong and Wu et al., 2021) and changing the population of ruminal microorganisms (Zhou et al., 2022), remains to be exploited.

Moreover, the lengths of the small intestine and total intestine were also affected by maternal feed restriction from early to mid-gestation. It is believed that the restriction at this stage is also more detrimental to this characteristic since there is evidence of a greater proportion of intestinal segment stretching during early gestation (Meyer et al., 2010). Thus, intestinal development becomes susceptible to changes caused by intrauterine conditions, influencing the use of nutrients by the offspring (Trahair et al., 1997; Godfrey and Barker, 2000; Wu et al., 2006; Wang et al., 2008).

The morphological results obtained in this study demonstrated differences in crypt depth and villus height:crypt depth ratio of newborns, depending on the stage of pregnancy in which the restriction occurs. The tendency of increased proliferation of the jejunal crypt in R-M animals corroborates another study conducted in cattle with nutritional restriction from early to mid-gestation (Meyer et al., 2010). The crypt depth is related to the proliferative potential of the enterocytes, moving in the apical direction of the villi, and the height of the intestinal villi indicates the number of enterocytes in the villi (Pluske et al., 1996; Bittrich et al., 2004). In addition, villus height is inversely associated with the proliferation rates of epithelial cells (Blättler et al., 2001; Sauter et al., 2004).

After birth, the jejunum must be able to digest and absorb the nutrients, processes considered important to sustain the high growth rate of the newborn (Tian et al., 2018). Brush border enzymes are responsible for the final stages of feed digestion by hydrolyzing macronutrients into smaller molecules to be absorbed (Tian et al., 2018). Any change in the activity of these enzymes can affect the absorption of nutrients and, consequently, the growth of the neonate. It has already been mentioned that the main carbohydrate present in the milk of most mammals is lactose and that high activities of this enzyme are found in the intestinal mucosa of newborns (Henning, 1985). In addition to lactase, other enzymes, including maltase and sucrase, degrade di- and oligosaccharides in monosaccharides, performing important functions to obtain energy for the animal (Huygelen et al., 2015). Some studies have reported that lactase activity declines with breastfeeding time (Manners and Stevens, 1972; Kelly et al., 1991) while maltase and sucrose activities increase at the end of this period (Kidder and Manners, 1980; Kelly et al., 1991). Therefore, the activities of maltase and sucrase are considered important markers of intestinal development (Huygelen et al., 2015; Pieper et al., 2016), since the increased activity of these enzymes indicates a faster maturation of the jejunal epithelium (Tian et al., 2018).

It is believed that the greater mRNA expression of lactase in the singletons of the R-M group was due to maternal refeeding in the second half of gestation. This phase is important due to the beginning of the process of intestinal maturation, that is when there is enzymatic secretion (Meyer and Caton, 2016).

Associated with maternal refeeding, single gestation also favored gene expression of this enzyme since it was previously verified that the competition for limited nutrients between the two fetuses of the same gestation could compromise the development of lambs, especially when there is a restriction in the second half of gestation (McCoard et al., 2000). Interestingly, there was no difference in *LCT* mRNA expression between the R-M (single) and M-R (twins) groups of the present study. There is no such approach for ruminants in the literature. However, research in prolific species, including rats and pigs, has already been performed to evaluate the activity of digestive enzymes. In a study conducted with intrauterine growth restricted (IUGR) rats, higher activity of the enzyme lactase and maltase was observed in the jejunum of these animals at birth compared with normal individuals (Qiu et al., 2005). The activity of the lactase enzyme was also higher at the jejunum border brush of newborn piglets when maternal malnutrition was observed during gestation (Cao et al., 2014). These studies have suggested that increased activity of these enzymes would be a form of adaptation to compensate for the lack of nutritional supply during prenatal life, increasing the digestive capacity of IUGR animals. Therefore, it is believed that this is the reason for restricted twins in the second half of gestation presenting *LCT* mRNA expression similar to the other offspring in the present study. Although it is unclear, it is believed that the similar expression of *LCT* as an adaptation mechanism in restricted twins in the second half of gestation is because this enzyme is the most important in early postnatal life for the digestion of lactose that is the main dietary carbohydrate (Trotta and Swanson, 2021). On the other hand, there is evidence that the activity of this enzyme in the small intestine of lambs decreases with age (Shirazi-Beechey et al., 1991). Moreover, the greater nutrient dispute between twin compared with singleton fetuses also explains the response related to the lower *MGAM* mRNA expression in fetuses of twin gestations.

To better understand how maternal feed restriction may influence intestinal growth and development, we also analyzed the expression of the gene encoding the receptor that binds to GLP-2. The GLP-2 is an endogenous regulatory peptide that possesses potent trophic activity on the intestinal mucosa (Lovshin et al., 2000; Tsai et al., 1997). In addition, GLP-2 is considered a modulator of the activity and expression of intestinal nutrient transporters, including SGLT1 (Cottrell et al., 2006) and GLUT2 (Au et al., 2002). The *SLC5A1* and *SLC2A2* are the encoding genes of SGLT1 and GLUT2, respectively. In newborn piglets, maternal nutritional restriction did not affect *GLP-2* receptor mRNA expression and SLC5A1 and SLC2A2 transporters compared with offspring whose dams received 100% of nutritional requirements during gestation (Cao et al., 2014). Similarly, no difference was observed in the *GLP-2* receptor mRNA expression in the offspring of the present study. However, the higher expression tendency of *SLC5A1* and *SLC2A2* mRNA in animals of the M-R group indicates that adequate nutritional intake during the first half of gestation seems to be indispensable to increasing the expression of these intestinal transporters at birth. Moreover, the fact that newborn twins present lower expression of *SLC2A2* mRNA, independent of maternal nutritional treatment during gestation, indicates that these animals may be more inefficient in absorbing monosaccharides in the postnatal phase.

Previously, it was found that nutritional restriction to the fetus may affect the development of the intestinal immune response function due to the increased risk of intestinal disorders (Han et al., 2013; Hu et al., 2015). During gestation in ruminants, the type of placental structure (epitheliochorial) prevents the transfer of serum immunoglobulins (Ig) from the dam to the fetus. Thus, the newborn is more immunologically susceptible to birth and totally dependent on the passive transfer of Ig through maternal colostrum to protect it against infections until its immune system is established. Hammer et al. (2011) reported that lambs whose dams had nutritional restriction during mid-to-late gestation were more efficient at absorbing Ig. When evaluating possible changes in intestinal permeability, it was observed that the animals of the R-M group of the present study presented lower expression of *OCLN* mRNA. This gene encodes the integral membrane protein, occludin, present in the tight junctions of epithelial cells and seems to be responsible for the formation of a barrier for macromolecules (Hu et al., 2015). Similarly, Zhang et al. (2018) found that IUGR lambs expressed less mRNA *ZO-1* and *OCLN* in the ileum compared with lambs considered normal. Thus, this may represent a possible efficiency acquired by the restricted offspring in the first half of gestation to absorb immunoglobulins at birth, since these animals had shorter intestines. However, high permeability of the intestinal epithelium also allows the entry of pathogens that can compromise the health of the offspring.

5. Conclusions

This study suggests that nutrient restriction during the first half of gestation is detrimental to the second half of gestation and cause more deleterious effects on the intestinal development and function (in terms of morphometry, morphology, and gene expression) of the offspring. Nevertheless, feed restriction applied during the early and mid-gestation was demonstrated to reduce the intestinal barrier function, which in turn may favor the absorption of immunoglobulins from colostrum, but also the access of pathogens to the offspring organism. Therefore, these findings reinforce the importance of nutrition care of pregnant dairy goats over gestation to avoid persistent effects on their offspring.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: L.R. Santos, M.S. Duarte and M.P. Gionbelli. Data curation: L.R. Santos, M.S. Duarte and M.P. Gionbelli. Formal analysis: T.C. Costa, R.O. Souza, T.R.S. Gionbelli, I.M. Oliveira Junior and M.P. Gionbelli. Funding acquisition: M.S. Duarte and M.P. Gionbelli. Investigation: L.R. Santos and M.S. Duarte. Methodology: L.R. Santos, T.C. Costa and I.M. Oliveira Junior. Project administration: M.S. Duarte and M.P. Gionbelli. Resources: M.S. Duarte and M.P. Gionbelli. Software: M.S. Duarte and M.P. Gionbelli. Supervision: M.S. Duarte and M.P. Gionbelli. Validation: L.R. Santos, M.S. Duarte and M.P. Gionbelli. Visualization: L.R. Santos, M.S. Duarte and M.P. Gionbelli. Writing – original draft: L.R. Santos. Writing – review & editing: L.R. Santos, T.C. Costa, T.R.S. Gionbelli, G.D. Ramírez-Zamudio, K.B. Nascimento, M.S. Duarte and M.P. Gionbelli.

Acknowledgments

The authors would like to acknowledge the agencies involved with the financing of this project – Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES; CAPES PROEX 676/2018 - grants number 23038.000839/2018-53 and #001), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; grant number APQ-02853-18), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grants number 315247/2020-7 and 309755/2017-4).

References

Au, A.; Gupta, A.; Schembri, P. and Cheeseman, C. I. 2002. Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. Biochemical Journal 367:247-254. https://doi.org/10.1042/bj20020393

Barcelos, S. S.; Nascimento, K. B.; Silva, T. E.; Mezzomo, R.; Alves, K. S.; Duarte, M. S. and Gionbelli, M. P. 2022. The effects of prenatal diet on calf performance and perspectives for fetal programming studies: a meta-analytical investigation. Animals 12:2145. https://doi.org/10.3390/ani12162145

Bittrich, S.; Philipona, C.; Hammon, H. M.; Romé, V.; Guilloteau, P. and Blum, J. W. 2004. Preterm as compared with full-term neonatal calves are characterized by morphological and functional immaturity of the small intestine. Journal of Dairy Science 87:1786-1795. https://doi.org/10.3168/jds.S0022-0302(04)73334-2

Blättler, U.; Hammon, H. M.; Morel, C.; Philipona, C.; Rauprich, A.; Romé, V.; Le Huërou-Luron, I.; Guilloteau, P. and Blum, J. W. 2001. Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. The Journal of Nutrition 131:1256-1263. https://doi.org/10.1093/jn/131.4.1256

Cao, M.; Che, L.; Wang, J.; Yang, M.; Su, G.; Fang, Z.; Lin, Y.; Xu, S. and Wu, D. 2014. Effects of maternal over-and undernutrition on intestinal morphology, enzyme activity, and gene expression of nutrient transporters in newborn and weaned pigs. Nutrition 30:1442-1447. https://doi.org/10.1016/j.nut.2014.04.016

Costa, T. C.; Moura, F. H.; Souza, R. O.; Lopes, M. M.; Fontes, M. M. S.; Serão, N. V. L.; Sanglard, L. P.; Du, M.; Gionbelli, M. P. and Duarte, M. S. 2019. 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. Animal Reproduction Science 206:46-59. https://doi.org/10.1016/j.anireprosci.2019.05.006

Costa, T. C.; Du, M.; Nascimento, K. B.; Galvão, M. C.; Meneses, J. A. M.; Schultz, E. B.; Gionbelli, M. P. and Duarte, M. S. 2021a. Skeletal muscle development in postnatal beef cattle resulting from maternal protein restriction during mid-gestation. Animals 11:860. https://doi.org/10.3390/ani11030860

Costa, T. C.; Gionbelli, M. P. and Duarte, M. S. 2021b. Fetal programming in ruminant animals: understanding the skeletal muscle development to improve meat quality. Animal Frontiers 11:66-73. https://doi.org/10.1093/af/vfab061

Costa, T. C.; Mendes, T. A. O.; Fontes, M. M. S.; Lopes, M. M.; Du, M.; Serão, N. V. L.; Sanglard, L. M. P.; Bertolini, F.; Rothschild, M. F.; Silva, F. F.; Gionbelli, M. P. and Duarte, M. S. 2021c. Transcriptome changes in newborn goats' skeletal muscle as a result of maternal feed restriction at different stages of gestation. Livestock Science 248:104503. https://doi.org/10.1016/j.livsci.2021.104503

Costa, T. C.; Dutra, L. L.; Mendes, T. A. O.; dos Santos, M. M.; Veroneze, R.; Gionbelli, M. P. and Duarte, M. S. 2022. Impact of maternal feed restriction at different stages of gestation on the proteomic profile of the newborn skeletal muscle. Animals 12:1011. https://doi.org/10.3390/ani12081011

Cottrell, J. J.; Stoll, B.; Buddington, R. K.; Stephens, J. E.; Cui, L.; Chang, X. and Burrin, D. G. 2006. Glucagon-like peptide-2 protects against TPN-induced intestinal hexose malabsorption in enterally refed piglets. American Journal of Physiology-Gastrointestinal and Liver Physiology 290:G293-G300. https://doi.org/10.1152/ajpgi.00275.2005

Cruz, W. F. G.; Schoonmaker, J. P.; Resende, F. D.; Siqueira, G. R.; Rodrigues, L. M.; Zamudio, G. D. R. and Ladeira, M. M. 2019. Effects of maternal protein supplementation and inclusion of rumen-protected fat in the finishing diet on nutrient digestibility and expression of intestinal genes in Nellore steers. Animal Science Journal 90:1200-1211. https://doi.org/10.1111/asj.13273

Desai, M.; Gayle, D.; Babu, J. and Ross, M. G. 2005. Permanent reduction in heart and kidney organ growth in offspring of undernourished rat dams. American Journal of Obstetrics and Gynecology 193:1224-1232. https://doi.org/10.1016/j.ajog.2005.05.041

Detmann, E.; Souza, M. A.; Valadares Filho, S. C.; Queiroz, A. C.; Berchielli, T. T.; Saliba, E. O. S.; Cabral, L. S.; Pina, D. S.; Ladeira, M. M. and Azevedo, J. A. G. 2012. Métodos para análise de alimentos. Suprema, Visconde do Rio Branco. 214p.

Duarte, M. S.; Gionbelli, M. P.; Paulino, P. V. R.; Serão, N. V. L.; Martins, T. S.; Tótaro, P. I. S.; Neves, C. A.; Valadares Filho, S. C.; Dodson, M. V.; Zhu, M. and Du, M. 2013. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. Livestock Science 153:60-65. https://doi.org/10.1016/j.livsci.2013.01.006

Fall, C. 2009. Maternal nutrition: effects on health in the next generation. Indian Journal of Medical Research 130:593-599.

Gionbelli, T. R. S.; Rotta, P. P.; Veloso, C. M.; Valadares Filho, S. C.; Carvalho, B. C.; Marcondes, M. I.; Ferreira, M. F. L.; Souza, J. V. F.; Santos, J. S. A. A.; Lacerda, L. C.; Duarte, M. S. and Gionbelli, M. P. 2017. Intestinal development of bovine foetuses during gestation is affected by foetal sex and maternal nutrition. Journal of Animal Physiology and Animal Nutrition 101:493-501. https://doi.org/10.1111/jpn.12572

Godfrey, K. M. and Barker, D. J. P. 2000. Fetal nutrition and adult disease. The American Journal of Clinical Nutrition 71:1344S-1352S. https://doi.org/10.1093/ajcn/71.5.1344s

Hales, C. N. and Barker, D. J. P. 2001. The thrifty phenotype hypothesis: type 2 diabetes. British Medical Bulletin 60:5-20. https://doi.org/10.1093/bmb/60.1.5

Hammer, C. J.; Thorson, J. F.; Meyer, A. M.; Redmer, D. A.; Luther, J. S.; Neville, T. L.; Reed, J. J.; Reynolds, L. P.; Caton, J. S. and Vonnahme, K. A. 2011. Effects of maternal selenium supply and plane of nutrition during gestation on passive transfer of immunity and health in neonatal lambs. Journal of Animal Science 89:3690-3698. https://doi.org/10.2527/jas.2010-3724

Han, F.; Hu, L.; Xuan, Y.; Ding, X.; Luo, Y.; Bai, S.; He, S.; Zhang, K. and Che, L. 2013. Effects of high nutrient intake on the growth performance, intestinal morphology and immune function of neonatal intra-uterine growth-retarded pigs. British Journal of Nutrition 110:1819-1827. https://doi.org/10.1017/S0007114513001232

Henning, S. J. 1985. Ontogeny of enzymes in the small intestine. Annual Review of Physiology 47:231-245.

Hong, H. T. and Wu, C. P. G. 2021. *Bacillus* spp.-fermented mixture in goat starter diets on growth performance, blood, and carcass and gastrointestinal traits. Revista Brasileira de Zootecnia 50:e20200242. https://doi.org/10.37496/rbz5020200242

Hu, L.; Liu, Y.; Yan, C.; Peng, X.; Xu, Q.; Xuan, Y.; Han, F.; Tian, G.; Fang, Z.; Lin, Y.; Xu, S.; Zhang, K.; Chen, D.; Wu, D. and Che, L. 2015. Postnatal nutritional restriction affects growth and immune function of piglets with intra-uterine growth restriction. British Journal of Nutrition 114:53-62. https://doi.org/10.1017/S0007114515001579

Huygelen, V.; De Vos, M.; Prims, S.; Vergauwen, H.; Fransen, E.; Casteleyn, C.; Van Cruchten, S. and Van Ginneken, C. 2015. Birth weight has no influence on the morphology, digestive capacity and motility of the small intestine in suckling pigs. Livestock Science 182:129-136. https://doi.org/10.1016/j.livsci.2015.11.003

Kelly, D.; Smyth, J. and McCracken, K. 1991. Digestive development of the early-weaned pig: 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. British Journal of Nutrition 65:169-180. https://doi.org/10.1079/BJN19910078

Keomanivong, F. E.; Lemley, C. O.; Camacho, L. E.; Yunusova, R.; Borowicz, P. P.; Caton, J. S.; Meyer, A. M.; Vonnahme, K. A. and Swanson, K. C. 2016. Influence of nutrient restriction and melatonin supplementation of pregnant ewes on maternal and fetal pancreatic digestive enzymes and insulin-containing clusters. Animal 10:440-448. https://doi.org/10.1017/S1751731115002219

Kidder, D. E. and Manners, M. J. 1980. The level and distribution of carbohydrases in the small intestine mucosa of pigs from 3 weeks of age to maturity. British Journal of Nutrition 43:141-153. https://doi.org/10.1079/BJN19800073

Lovshin, J.; Yusta, B.; Iliopoulos, I.; Migirdicyan, A.; Dableh, L.; Brubaker, P. L. and Drucker, D. J. 2000. Ontogeny of the glucagon-like peptide-2 receptor axis in the developing rat intestine. Endocrinology 141:4194-4201. https://doi.org/10.1210/endo.141.11.7773

Manners, M. and Stevens, J. 1972. Changes from birth to maturity in the pattern of distribution of lactase and sucrase activity in the mucosa of the small intestine of pigs. British Journal of Nutrition 28:113-127. https://doi.org/10.1079/BJN19720014

McCoard, S. A.; McNabb, W. C.; Peterson, S. W.; McCutcheon, S. N. and Harris, P. M. 2000. Muscle growth, cell number, type and morphometry in single and twin fetal lambs during mid to late gestation. Reproduction, Fertility and Development 12:319-327. https://doi.org/10.1071/RD99059

McMillen, I. C.; Adams, M. B.; Ross, J. T.; Coulter, C. L.; Simonetta, G.; Owens, J. A.; Robinson, J. S. and Edwards, L. J. 2001. Fetal growth restriction: adaptations and consequences. Reproduction 122:195-204. https://doi.org/10.1530/rep.0.1220195

Meyer, A. M. and Caton, J. S. 2016. Role of the small intestine in developmental programming: impact of maternal nutrition on the dam and offspring. Advances in Nutrition 7:169-178. https://doi.org/10.3945/an.115.010405

Meyer, A. M.; Reed, J. J.; Vonnahme, K. A.; Soto-Navarro, S. A.; Reynolds, L. P.; Ford, S. P.; Hess, B. W. and Caton, J. S. 2010. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. Journal of Animal Science 88:2410-2424. https://doi.org/10.2527/jas.2009-2220

Nascimento, K. B.; Galvão, M. C.; Meneses, J. A. M.; Moreira, G. M.; Ramírez-Zamudio, G. D.; Souza, S. P.; Prezotto, L. D.; Chalfun, L. H. L.; Duarte, M. S.; Casagrande, D. R. and Gionbelli, M. P. 2022. Effects of maternal protein supplementation at mid-gestation of cows on intake, digestibility, and feeding behavior of the offspring. Animals 12:2865. https://doi.org/10.3390/ani12202865

NRC - National Research Council. 2001. Nutrient requirements of dairy cattle. 7th ed. The National Academies Press, Washington, DC, USA.

NRC - National Research Council. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. The National Academies Press, Washington, DC.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research 29:e45. https://doi.org/10.1093/nar/29.9.e45

Pieper, R.; Scharek-Tedin, L.; Zetzsche, A.; Röhe, I.; Kröger, S.; Vahjen, W. and Zentek, J. 2016. Bovine milk–based formula leads to early maturation-like morphological, immunological, and functional changes in the jejunum of neonatal piglets. Journal of Animal Science 94:989-999. https://doi.org/10.2527/jas.2015-9942

Pluske, J. R.; Williams, I. H. and Aherne, F. X. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Animal Science 62:131-144. https://doi.org/10.1017/S1357729800014417

Qiu, X. S.; Huang, T. T.; Shen, Z. Y.; Deng, H. Y. and Ke, Z. Y. 2005. Effect of early nutrition on intestine development of intrauterine growth retardation in rats and its correlation to leptin. World Journal of Gastroenterology 11:4419-4422. https://doi.org/10.3748/wjg.v11.i28.4419

Reed, J. J.; Ward, M. A.; Vonnahme, K. A.; Neville, T. L.; Julius, S. L.; Borowicz, P. P.; Taylor, J. B.; Redmer, D. A.; Grazul-Bilska, A. T.; Reynolds, L. P. and Caton, J. S. 2007. Effects of selenium supply and dietary restriction on maternal and fetal body weight, visceral organ mass and cellularity estimates, and jejunal vascularity in pregnant ewe lambs. Journal of Animal Science 85:2721-2733. https://doi.org/10.2527/jas.2006-785

Rodrigues, L. M.; Schoonmaker, J. P.; Resende, F. D.; Siqueira, G. R.; Machado Neto, O. R.; Gionbelli, M. P.; Gionbelli, T. R. S. and Ladeira, M. M. 2020. Effects of protein supplementation on Nellore cows' reproductive performance, growth, myogenesis, lipogenesis and intestine development of the progeny. Animal Production Science 61:371-380. https://doi.org/10.1071/AN20498

Santos, M. M.; Costa, T. C.; Ramírez-Zamudio, G. D.; Nascimento, K. B.; Gionbelli, M. P. and Duarte, M. S. 2022. Prenatal origins of productivity and quality of beef. Revista Brasileira de Zootecnia 51:e20220061. https://doi.org/10.37496/RBZ5120220061

Sauter, S. N.; Roffler, B.; Philipona, C.; Morel, C.; Romé, V.; Guilloteau, P.; Blum, J. W. and Hammon, H. M. 2004. Intestinal development in neonatal calves: Effects of glucocorticoids and dependence on colostrum feeding. Biology of the Neonate 85:94-104. https://doi.org/10.1159/000074965

Shirazi-Beechey, S. P.; Smith, M. W.; Wang, Y. and James, P. S. 1991. Postnatal development of lamb intestinal digestive enzymes is not regulated by diet. The Journal of Physiology 437:691-698. https://doi.org/10.1113/jphysiol.1991.sp018619

Soto-Navarro, S. A.; Lawler, T. L.; Taylor, J. B.; Reynolds, L. P.; Reed, J. J.; Finley, J. W. and Caton, J. S. 2004. Effect of high-selenium wheat on visceral organ mass, and intestinal cellularity and vascularity in finishing beef steers. Journal of Animal Science 82:1788-1793. https://doi.org/10.2527/2004.8261788x

Tedeschi, L. O.; Cannas, A. and Fox, D. G. 2010. A nutrition mathematical model to account for dietary supply and requirements of energy and other nutrients for domesticated small ruminants: the development and evaluation of the Small Ruminant Nutrition System. Small Ruminant Research 89:174-184. https://doi.org/10.1016/j.smallrumres.2009.12.041

Tian, S.; Wang, J.; Yu, H.; Wang, J. and Zhu, W. 2018. Effects of galacto-oligosaccharides on growth and gut function of newborn suckling piglets. Journal of Animal Science and Biotechnology 9:75. https://doi.org/10.1186/s40104-018-0290-9

Trahair, J. F. and Sangild, P. T. 2002. Chapter 1 Studying the development of the small intestine: philosophical and anatomical perspectives. p.1-54. In: Biology of growing animals. Vol. 1. Elsevier. https://doi.org/10.1016/S1877-1823(09)70117-1

Trahair, J. F.; DeBarro, T. M.; Robinson, J. S. and Owens, J. A. 1997. Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. The Journal of Nutrition 127:637-641. https://doi.org/10.1093/jn/127.4.637

Trotta, R. J. and Swanson, K. C. 2021. Prenatal and postnatal nutrition influence pancreatic and intestinal carbohydrase activities of ruminants. Animals 11:171. https://doi.org/10.3390/ani11010171

Tsai, C. H.; Hill, M.; Asa, S. L.; Brubaker, P. L. and Drucker, D. J. 1997. Intestinal growth-promoting properties of glucagon-like peptide-2 in mice. American Journal of Physiology-Endocrinology and Metabolism 273:E77-E84. https://doi.org/10.1152/ajpendo.1997.273.1.E77

Vonnahme, K. A. 2007. Nutrition during gestation and fetal programming. In: Range Beef Cow Symposium XX. University of Nebraska Lincoln, Fort Collins, Colorado.

Wang, J.; Chen, L.; Li, D.; Yin, Y.; Wang, X.; Li, P.; Dangott, L. J.; Hu, W. and Wu, G. 2008. Intrauterine growth restriction affects the proteomes of the small intestine, liver, and skeletal muscle in newborn pigs. The Journal of Nutrition 138:60-66. https://doi.org/10.1093/jn/138.1.60

Wu, G.; Bazer, F. W.; Wallace, J. M. and Spencer, T. E. 2006. Board-invited review: Intrauterine growth retardation: implications for the animal sciences. Journal of Animal Science 84:2316-2337. https://doi.org/10.2527/jas.2006-156

Zhang, H.; Sun, L.; Wang, Z.; Deng, M.; Nie, H.; Zhang, G.; Ma, T. and Wang, F. 2016. N-carbamylglutamate and L-arginine improved maternal and placental development in underfed ewes. Reproduction 151:623-635. https://doi.org/10.1530/REP-16-0067

Zhang, H.; Zhao, F.; Peng, A.; Dong, L.; Wang, M.; Yu, L.; Loor, J. J. and Wang, H. 2018. Effects of dietary L-arginine and *N*-carbamylglutamate supplementation on intestinal integrity, immune function, and oxidative status in intrauterine-growth-retarded suckling lambs. Journal of Agricultural and Food Chemistry 66:4145-4154. https://doi.org/10.1021/acs.jafc.8b00726

Zhou, X.; Yang, H.; Yan, Q.; Ren, A.; Kong, Z.; Tang, S.; Han, X.; Tan, Z. and Salem, A. Z. M. 2019. Evidence for liver energy metabolism programming in offspring subjected to intrauterine undernutrition during midgestation. Nutrition & Metabolism 16:20. https://doi.org/10.1186/s12986-019-0346-7

Zhou, W.; Wu, X.; Su, C.; Li, L.; Xu, L.; Akhtar, R. W.; Shah, S. A. H. and Chen, H. 2022. Exploring the rumen microbial community in Guizhou White goats at different ages. Revista Brasileira de Zootecnia 51:e20210070. https://doi.org/10.37496/rbz5120210070

Zi, Y.; Ma, C.; Yang, Y.; Zhao, L.; Liu, Y. and Gao, F. 2023. Oxidative damage and exocrine dysfunction of ovine fetal pancreas are induced by maternal nutritional restriction. Revista Brasileira de Zootecnia 52:e20220088. https://doi.org/10.37496/rbz5220220088