LUANA RUIZ DOS SANTOS

DEVELOPMENT OF GASTROINTESTINAL TRACT OF NEWBORN GOATS UNDER MATERNAL FEED RESTRICTION AT DIFFERENT STAGES OF GESTATION

LAVRAS-MG
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Master's dissertation presented to the Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Animal Science, area of concentration in Production and Nutrition of Ruminants, to obtain the title of Master.

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Approved in April 18, 2019.

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À minha família, em especial, à minha mãe,
Fátima, pelo apoio, incentivo, compreensão e
por ser o meu maior exemplo de vida.
Dedico

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ABSTRACT

The objective of this study was to elucidate the effect of maternal feed restriction during two distinct phases of gestation (initial half and final half) on the formation of vital organs and growth and development of the small intestine of the offspring. Fourteen pregnant goats were divided into two treatments with different dietary levels, according to the gestation phase. Eight of them were fed 100% of total digestible nutrients (TDN) and crude protein (CP), according to the recommendations of the NRC (2007), in the first half of pregnancy and then received 50% of TDN and CP until parturition date (M-R treatment). The remaining six goats were fed 50% of TDN and CP in the first half of pregnancy and received 100% of TDN and CP in the second half of pregnancy (R-M treatment). Male offspring was separated from the dams at birth and blood samples were collected from these animals. Later, the newborn goats were weighed and slaughtered for collection of corporal components. Maternal feed restriction did not affect the blood glucose concentration, birth weight, organ weights and other body components of the slaughtered offspring ($P > 0.13$). The weight of reticulum-rumen and omasum did not differ between treatments ($P = 0.893$), but tended to be heavier ($P = 0.057$) in the M-R group, when expressed per kg of body weight. The small intestine ($P = 0.055$) and total intestine ($P = 0.095$) tended to be heavier in the offspring of the M-R group, and this characteristic was more pronounced ($P = 0.038$) when expressed in kg body weight. The lengths of the small intestine and total intestine were higher ($P \leq 0.05$) in the M-R group, however, no differences were observed in the weight to length ratio of the small intestine and total intestine of the offspring ($P > 0.46$). Maternal feed restriction also did not influence the height of the intestinal villi ($P = 0.406$). However, newborn goats of group R-M had lower villus height to crypt depth ratio ($P = 0.016$), due to the tendency of the greater of crypt depth in these animals ($P = 0.081$). No effect of maternal feed treatment was observed in the mRNA expression of the MGAM and GLP-2R in the jejunum of the offspring ($P > 0.12$), but 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 ($P = 0.061$) and this effect was more pronounced in the SLC2A2 mRNA expression ($P = 0.025$), compared to the animals from the twin gestation. In addition, a maternal feed restriction × number of fetuses interaction was observed for LCT mRNA expression ($P = 0.043$). Singletons of the R-M treatment expressed more LCT mRNA compared to the offspring of the M-R (single) and R-M (twin) groups, but no difference was observed when compared to the twins of the M-R group. In general, the results obtained in this study demonstrated that maternal feed restriction at different stages of gestation alters differently the growth and development of the small intestine. Even without changes in body weight and blood glucose levels of newborns, restriction in the first half of gestation may be more detrimental to the performance and health of offspring throughout life, due to increased impairment of intestinal development.

Key-words: Fetal programming, intestine, organogenesis, ruminants

RESUMO

O objetivo com este estudo foi elucidar o efeito da restrição alimentar materna durante duas fases distintas da gestação (metade inicial e metade final) sobre a formação de órgãos vitais e crescimento e desenvolvimento do intestino delgado da prole. Quatorze cabras gestantes foram divididas em dois tratamentos com níveis dietéticos distintos, conforme a fase da gestação. Oito delas foram alimentadas com 100% dos nutrientes digestíveis totais (NDT) e proteína bruta (PB) de acordo com as recomendações do NRC (2007), na primeira metade da gestação e depois passaram a receber 50% de NDT e PB até a data do parto (tratamento M-R). As seis cabras restantes foram alimentadas com 50% de NDT e PB na primeira metade da gestação e depois receberam 100% de NDT e PB na segunda metade da gestação (tratamento R-M). A prole masculina foi separada das mães ao nascimento e amostras de sangue foram coletadas desses animais. Posteriormente, as cabras recém-nascidas foram pesadas e abatidas para coleta de componentes corporais. A restrição alimentar materna não afetou a glicemia, peso ao nascer, pesos dos órgãos e outros componentes corporais avaliados na prole abatida ($P > 0,13$). O peso do retículo-rúmen e omaso não diferiu entre os tratamentos ($P = 0,893$), porém tendeu a ser mais pesado ($P = 0,057$) no grupo M-R, quando expresso por kg de peso corporal. O intestino delgado ($P = 0,055$) e o intestino total ($P = 0,095$) tenderam a ser mais pesados na prole do grupo M-R e essa característica foi mais pronunciada ($P = 0,038$), quando expressa em kg de peso corporal. Os comprimentos de intestino delgado e intestino total foram maiores ($P \leq 0,05$) no grupo M-R, entretanto não foram observadas diferenças na relação peso por comprimento do intestino delgado ou peso por comprimento do intestino total da prole ($P > 0,46$). A restrição alimentar materna também não influenciou a altura das vilosidades intestinais ($P = 0,406$). No entanto, as cabras recém-nascidas do grupo R-M apresentaram menor relação entre a altura das vilosidades e a profundidade das criptas ($P = 0,016$), devido à tendência de maior profundidade das criptas nesses animais ($P = 0,081$). Não foi observado efeito do tratamento alimentar materno na expressão de mRNA dos genes MGAM e GLP-2R no jejuno da prole ($P > 0,12$), porém os recém-nascidos do grupo M-R tenderam a expressar mais mRNA dos genes SLC5A1 ($P = 0,091$), SLC2A2 ($P = 0,091$) e OCLN ($P = 0,061$). Os animais nascidos de gestação simples, tenderam a expressar mais mRNA do gene MGAM ($P = 0,061$) e esse efeito foi mais pronunciado na expressão de mRNA do gene SLC2A2 ($P = 0,025$), comparado aos animais oriundos de gestação gemelar. Além disso, observou-se uma interação restrição alimentar materna × número de fetos para a expressão de mRNA da LCT ($P = 0,043$). Filhos únicos do tratamento com R-M expressaram mais mRNA da LCT em comparação com os descendentes dos grupos M-R (filhos únicos) e R-M (gêmeos), mas nenhuma diferença foi observada quando comparados aos gêmeos do grupo M-R. Em geral, os resultados obtidos neste estudo demonstraram que a restrição alimentar materna em estágios distintos de gestação altera diferentemente o crescimento e desenvolvimento do intestino delgado. Mesmo sem haver alterações no peso corporal e níveis de glicose no sangue dos recém-nascidos, a restrição na primeira metade da gestação pode ser mais prejudicial à saúde e ao desempenho da prole ao longo da vida, devido ao maior comprometimento do desenvolvimento intestinal.

Palavras-chave: Intestino, organogênese, programação fetal, ruminantes.

DESENVOLVIMENTO DO TRATO GASTROINTESTINAL DE CABRAS RECÉM-NASCIDAS SOB RESTRIÇÃO ALIMENTAR MATERNA EM DIFERENTES ESTÁGIOS DE GESTAÇÃO

No Brasil, grande parte da produção de ruminantes é realizada em sistemas extensivos. Diante disso, as variações climáticas fazem com que a disponibilidade de forragem oscile durante o ano. O objetivo deste estudo foi avaliar se o crescimento e o desenvolvimento intestinal de cabritos alteram de acordo com a fase de restrição alimentar materna. Além disso, foi avaliado se existe um estágio de gestação que a restrição é mais prejudicial ao desenvolvimento da progênie. Dois grupos de cabras prenhes receberam a mesma quantidade de alimento, fornecida em dois períodos diferentes (primeira ou segunda metade da gestação). Os componentes corporais dos recém-nascidos foram analisados. Em geral, os resultados obtidos neste estudo demonstraram que a restrição alimentar materna na primeira metade da gestação pode ser mais prejudicial ao desenvolvimento intestinal. Portanto, deve-se garantir alimentos para cabras gestantes, especialmente nesta fase para não comprometer a saúde e o desempenho da progênie até a idade adulta.

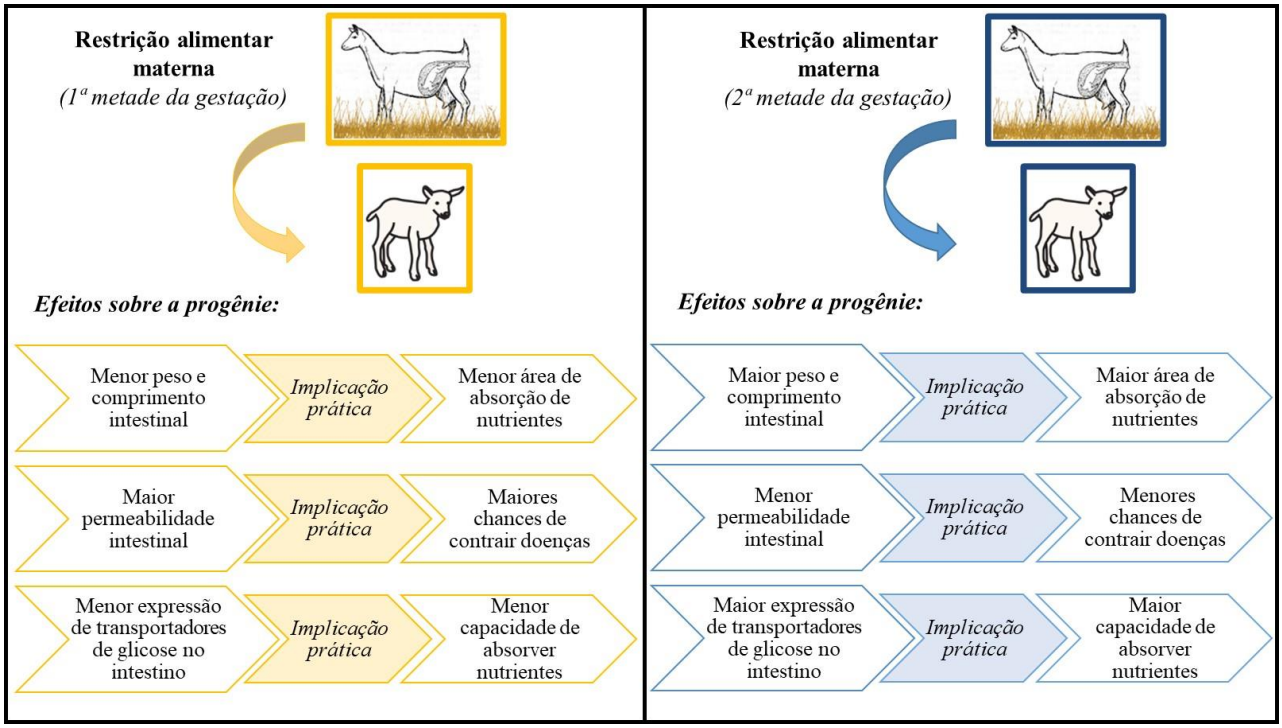

Figura 1. Efeitos da restrição alimentar materna (primeira ou segunda metade da gestação) ou número de fetos (únicos ou gêmeos) em diversos processos de crescimento e desenvolvimento do intestino de cabras recém-nascidas.

Dissertação de mestrado em Zootecnia na UFLA, defendida em 18 de abril de 2019.

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SUMMARY

1 INTRODUCTION

In general, sheep and goats have reproductive seasonality, mainly due to variations in nutritional level and the photoperiod, which is accentuated in temperate regions or with high altitudes and latitudes. It is believed that these animals have developed this ability due to natural evolution. In order to guarantee the most favorable season for progeny growth and development, evolutionary adaptation has programmed births and lactation for a favorable time of year (Wayne et al., 1989), usually in early spring, when the availability and feed quality increases. This is a natural selection question, because if births occurred in the fall or winter, there would be high chances of mortality of the offspring. Thus, these animals began to develop mechanisms to recognize the decrease or increase in the number of daily hours, by which they regulate reproduction (Rosa and Bryant, 2003), becoming sexually active often from late summer to early winter. Therefore, important stages are impaired until the mid-gestation, when they coincide with the dry season of the year due to feed shortages.

Environmental effects in the early stages of an animal's life, especially in the uterus, not only affect its development but also its health and performance throughout its life (Barker et al., 1993). This has been an important study area of study in nutrition, physiology, genetics and human and animal epidemiology research, being referred to as Fetal Programming (Barker, 1997) or Developmental Programming (Reynolds et al., 2010).

Fetal growth and development can be affected by several factors and inadequate maternal nutrition is the main cause that compromises the supply of nutrients to the fetus (Reynolds et al., 2010). The tissues formation, organs, gastrointestinal tract (GIT), especially the small intestine, can be programmed during the prenatal phase. Growth, development, and vascularization of the small intestine are essential processes that ensure immunological protection, nutrient metabolism, neonatal survival, and postnatal growth. It is a dynamic tissue, capable of changing or adapting to variation of the diet, physiological state or environment in which the animal is subjected (Trahair and Sangild, 2002).

Although some studies have evaluated the consequences of maternal malnutrition on the growth and development parameters of the fetal small intestine in ruminants, there are some post-transcriptional gaps to be analyzed. To our knowledge, there are no previous studies that emphasized the mRNA expression pattern of growth factors, tight junction proteins, digestive enzymes and glucose transporters in the small intestine of newborn ruminants, under maternal

feed restriction during phases different from gestation. In addition, the effects of feeding levels according to the gestational periods on fetal development, should be understood for the elaboration of feeding programs based on the best time of allocation of feed resources to optimize production efficiency. Based on the analysis of previous data in the literature and the existing knowledge gaps, we tested the hypothesis that maternal feed restriction at distinct moments of gestation alters differently the growth and development of the small intestine of newborns. Therefore, this study had the objectives to elucidate the effect of maternal feed restriction in two distinct gestation stages (first or second half) on the formation of organs, growth and development of the small intestine and regulatory mechanisms of the intestinal barrier function, digestion of disaccharides and glucose uptake in newborn goats.

2 BACKGROUND

2.1 Physiological mechanisms and reproductive seasonality

From a reproductive point of view, sheep and goats are considered "short day" seasonal poliestral animals, as they show successive estrus due to decrease of daytime hours (Rosa and Bryant, 2003). This mechanism is due to lower light incidence on the retina of the eyes, causing the optic nerve to promote a nervous stimulating on the pineal gland, which is responsible for melatonin production. Melatonin is a hormone that acts on GnRH (gonadotropin releasing hormone) release by the hypothalamus. This, in turn, stimulates the FSH and LH secretion (follicle stimulating hormone and luteinizing hormone, respectively) by the pituitary gland (Barrell et al., 1992). In equatorial and tropical regions, changes in day period are less pronounced, thus, the reproductive seasonality is less evident, allowing the reproduction of these animals throughout the year and presenting relatively short postpartum anestrus. However, the beginning and duration of reproductive season of goats depend not only on the photoperiod, but also on a number of factors related to nutrition, climate, genetics, physiological stage and others.

Thus, evolutionarily, the most propitious time to initiate the reproductive activity that these animals found, in order to guarantee the survival of the progeny, is between fall and winter. However, when the gestation of goats begins in late summer or early fall, it is expected that the availability of feed during pregnancy (average duration of 150 d) will decline until just

before parturition (early spring) due to adverse climatic conditions (dry season of the year). This is the major problem faced, since the nutritional requirements of the matrices increase in the course of gestation.

Therefore, it is necessary to be attentive to the nutritional plan of pregnant females, providing feed resources at the period of lower feed supply, in order not to prejudice the development of the progeny. In addition, it is extremely important to carry out the breeding season according to the region, for better productivity of the herd and planning the products supply period to the consumer market.

2.2 Maternal nutritional status *vs.* fetal growth and development

Studies have evaluated maternal nutritional restriction on fetal growth and development and its effects throughout the progeny's life. In cattle research, for example, it was found that there is a higher risk of mortality and morbidity in animals born from malnourished females during gestation, including intestinal and respiratory dysfunctions, compromising their growth (Wu et al., 2006).

The feed shortage during gestation can lead to competition for nutrients between the fetus and the other maternal metabolic processes, simultaneously with gestation. This is more evident in young heifers, since they are still in the growing phase, that is, the nutrients acquired through diet will be destined both for the maintenance of physiological processes of the pregnant female and for fetal growth and development. Thus, what will define the ability of the pregnant female to generate a new individual is mainly the way she will partition the nutrients to enable embryonic, placental and fetal development, concomitantly to their own requirements. Much information about how this nutrients redistribution occurs and how they work in the maternal and fetal organism comes from research with small ruminants (sheep and goats), probably, because of the reduced cost and easier study.

According to Hammond (1947), the pregnant females fractionate the pool of available nutrients in order to favor the progeny. This same author proposed that different tissues compete for circulating nutrients, according to their respective metabolic rates. However, further studies revealed that rather than competition, there is a mechanism of endocrine regulation of tissues. Thus, arose the concept of "homeorhesis" developed by Bauman and Currie (1980), in which several tissues influence the metabolism at the same time, so all events that occur in the maternal

organism are attended in a more coherent way. The purpose of this metabolic control is to optimize fetal growth and, consequently, its postnatal survival, reducing the chances of drastic depletion of maternal body reserves.

During the initial third of gestation, the nutrients demand for embryonic growth is low compared to more advanced stages. However, it is extremely important to consider this phase due to the critical events that start on it (establishment of functional fetal circulation and uteroplacental circulation, organogenesis, myogenesis and adipogenesis), for the complete development of the fetus (Du et al., 2010). Thus, the female should be in a positive energy balance at this stage, becoming more able to support the fetus, without causing any major damage to the pregnant female and descendant(s). The last third of gestation is the phase of greater nutritional demand for fetal growth, since it is when approximately 75% of fetal ruminant growth occurs (Funston et al., 2010). In cattle, this period often coincides with the dry period of the year, since the lack of nutritional contribution to the fetus may affect the birth weight of the animal, with a high probability of affecting the growth and health of the offspring, long-term (Robinson, 1977). In small ruminants, depending on the period of the breeding season, fetal growth during the last stage of pregnancy may or may not be affected, because the gestation time is relatively shorter compared to cattle's.

Although most studies of fetal programming emphasize the effects of maternal nutritional restriction on offspring (Kotsampasi et al., 2009; Long et al., 2010), factors such as maternal size and litter size also affect the intrauterine environment, influencing fetal development and consequently, neonatal performance (Kenyon and Blair, 2014).

2.2.1 Nutritional requirement and intrauterine metabolism

In the last third of gestation, accelerated fetal growth occurs simultaneously with the development of other elements such as placenta, fetal fluid, fetal membranes and uterine support tissues, which leads to requirement of large concentrations of glucose and amino acids by the gravid uterus. Glucose is essential for fetal and placental metabolism in all mammalian species already studied. In ruminants, glucose synthesis occurs from propionate through hepatic gluconeogenesis. This, in turn, is a volatile fatty acid obtained by the fermentation of carbohydrates of fast degradability in the rumen, absorbed by the ruminal papillae, directed to the liver and metabolized later.

In Brazil, the predominant production system is pasture, being the animals feeding composed only by bulky feed, most of the times, which leads to greater ruminal acetate proportion in relation to the other volatile fatty acids. Thus, the glucose synthesis in the liver decreases, since there is a smaller amount of propionate produced and this causes the animal's organism, especially in the last third of gestation, to seek alternatives to obtain glucose in order to meet fetoplacental requirements.

Some metabolic patterns models have been developed for gestation in sheep (Bell et al., 1993), cows (Bell, 1995) and in general (Bell et al., 2005), in which it is assumed that in the last third of gestation in both species, 35 to 40% of fetal energy is obtained through glucose and lactate, and about 55% is supplied as amino acids. Acetate provides only part of the remaining 5 to 10%, contributing a very small share compared to its relative abundance and importance to the maternal system.

In general, ruminants have difficulty raising circulating glucose levels, and the use of amino acids as precursors for gluconeogenesis is probably the easiest way to meet the high demand for glucose. To meet this requirement, mobilization of maternal tissues occurs in order to support gluconeogenesis and amino acid requirements of the fetus as gestation time increases. In addition to the mobilization of lean tissue, fat mobilization occurs through the maternal organism, but the placental capacity to transport long chain non-esterified fatty acids (NEFA) and ketone bodies (Bell et al., 2005) is extremely limited. This fact makes impossible for the fetus to use these substrates to meet their energy needs, and the released NEFA are destined, especially to obtain maternal energy, in order to save glucose and amino acids to the fetus. (Bell and Ehrhardt, 2000).

Therefore, in addition to metabolism and fetal growth, the other non-fetal components, such as the placenta mainly, influence the nutrients partition destined for the gravid uterus and, consequently, the nutritional requirements.

2.2.2 Gestational nutrition and fetal organogenesis

The formulation of maternal diet during gestation can influence the viability and body composition of the individual being generated (Symonds et al., 2010). In addition, the colostrum intake in neonatal animals is essential for obtaining passive immunity and energy (Blum, 2006).

In view of this, the complete growth and development of GIT is essential to animal survival, as this is the main place of nutrients absorption and immunoglobulins of the newborn.

Studies on fetal programming with emphasis on the development of GIT have arisen from epidemiological surveys done by human medicine since the 1960s. In these studies, it was found that children under intrauterine growth retardation (IUGR) were more likely to acquire infections after birth (Gruenwald, 1963) and that the immune system was more vulnerable in low birth weight individuals (Watson et al., 1979). Although it is possible to repair body mass growth through postnatal nutrition, it can still be compromised, given that certain changes in GIT functions are not reestablished, such as epithelial permeability (Lunn et al., 1991). In studies with sheep, nutritional and oxygen shortages for a prolonged period repressed fetal growth (Harding et al., 1985) and caused damage to the development of GIT, especially the small intestine (Avila et al., 1989).

In ruminants, most fetal growth occurs during the last third of gestation, while organogenesis occurs during the first half of gestation (Redmer et al., 2004; Fowden et al., 2006; Vonnahme et al., 2007; Caton et al., 2009; Meyer et al., 2010). According to Weaver et al. (1991), it is also in the last third of gestation that there is greater growth of GIT in human, bovine and ovine species. However, maternal nutritional restriction during the last gestation phase may not be the only triggering factor for abnormal fetal GIT development, but we need to consider also the scarcity of nutritional intake during the first half of gestation.

Trahair et al. (1997) evaluated maternal nutritional restriction in the first half of pregnancy in sheep and did not observe significant differences in total weight and growth of fetal organs. However, the GIT weight was lower in restricted group compared to control group, as it impaired the development of the small and large intestines of the fetuses, presenting a smaller diameter and intestinal mucosal area.

Meyer et al. (2010) analyzed the feed restriction of pregnant cows up to gestational day 125 and fed them up to gestational day 245, in order to reach a body condition score similar to that of the control group, which was not restricted. These authors reported that there was a higher cell proliferation in the jejunum of the fetuses under feed restriction up to gestational day 125 and greater total jejunal vascularization up to gestational day 245. These facts indicate that the fetal intestine was adapting and becoming more efficient during maternal nutritional restriction, according to the "thrifty phenotype" hypothesis (Hales and Barker, 1992), which resulted in higher tissue growth and vascularization in the period in which the dams were

realimented. In addition, animals born from sheep malnourished during pregnancy are more efficient in absorbing immunoglobulins from colostrum (Hammer et al., 2011), because maternal nutritional restriction results in low colostrum production, but with immunoglobulin concentration similar to that of sheep receiving adequate nutrition during gestation. (Swanson et al., 2008).

However, in a study carried out in Brazil, pregnant cows (Holstein x Gir) received two nutritional levels (maintenance with restricted consumption x *ad libitum*) and no differences were observed in the development of fetal GIT during pregnancy (Gionbelli et al., 2016). Thus, the fetal gut adaptation effect for greater immunoglobulin uptake may occur only in breeds and/or species whose dams produce milk and/or colostrum in smaller amounts, such as specialized breeds for meat production.

Studies on organogenesis, development of the GIT, and effect of fetal programming throughout the progeny's life are still inconclusive. Therefore, we need frequent research to understand better, how maternal malnutrition influences the development of organs, fetal tissues and, consequently, the postnatal performance of progeny.

2.2.3 Effects of fetal programming on small intestine growth and development

According to Crosnier et al. (2006) the intestinal epithelium is most renewable tissue compared to the other tissues in adult mammals. It is formed during gastrulation, from the endoderm and remains as a stratified cuboidal epithelium until mid-gestation in most vertebrates. (De Santa Barbara et al., 2003). One of the main morphological characteristics of the small intestine is the presence of contiguous villi and crypts in its structure (Figure 2). Both are formed from epithelial mesenchymal interactions during the prenatal phase. The villi contain three types of differentiated cells: enterocytes (absorptive cells), goblet cells (cells secreting a protective mucus barrier) and enteroendocrine cells (cells that release gastrointestinal hormones). All of them have the characteristics of mature epithelial cells. Crypts are invaginations of the epithelium in the intestinal mucosa, mainly composed of intestinal stem cells (ISCs), but they present another type of differentiated cells at its base, the Paneth cells, that secreting antibacterial peptides (Cheng and Leblond, 1974). The ISCs are responsible for promoting the constant renewal of the cellular population of the intestinal epithelium. As the cells begin to differentiate, they migrate towards the intestinal lumen (Figure

2). This process is accompanied by a reduction of proliferative rhythm and apoptosis at the apex of the villi (Jiang and Edgar, 2012; Umar, 2010). In summary, the crypt is the proliferative, monoclonal compartment and is maintained by multipotent stem cells, while the villi represents the differentiated compartment and is polyclonal because it receives cells from multiple crypts (Wright, 2000).

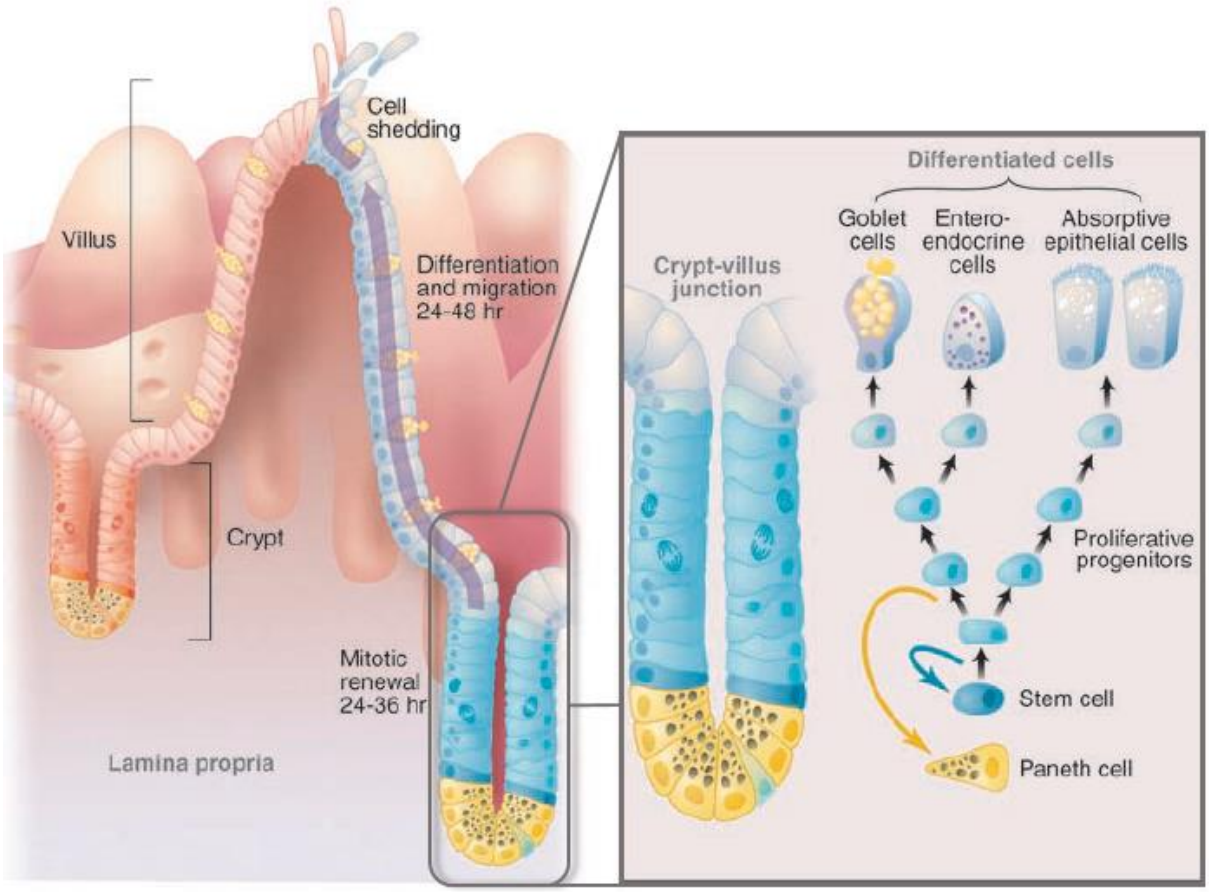

Figure 2. Anatomy of intestinal epithelium and cell differentiation from stem cells. Adapted from Radtke and Clevers (2005)

Growth and development of the small intestine occur in the fetal, perinatal and neonatal phases (Nathanielsz, 2006; Symonds et al., 2007; Fowden, 2006; Meyer and Caton, 2016) In addition, it continues to develop in the postnatal period until it reaches maturity, as it remains responsive to several conditions. The presence of nutrients in the lumen, hormones, microorganisms and physiological stage are some of the factors that lead to changes in intestinal characteristics (Trahair and Sangild, 2002).

The Figure 3 represents the growth and development stages of small intestine and the major influences on it throughout the animal's life.

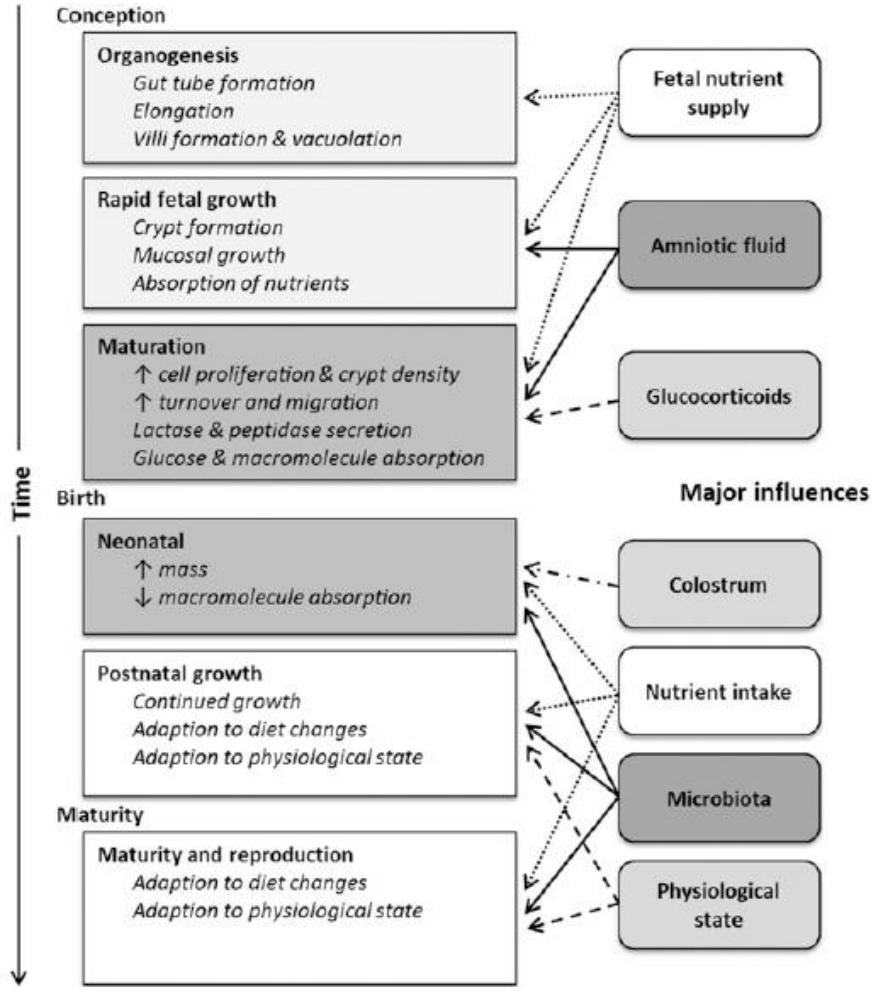

Figure 3. Growth and development windows of the small intestine and regulatory factors. (Meyer and Caton, 2016).

In the literature, there are studies that investigated the influence of maternal nutritional restriction on the growth and development of small intestine. Fetuses from sheep with access to restricted diet until the middle third of gestation did not present differences in the small intestine mass compared to animals that were not restricted. This indicates that nutrient deprivation during these phases may not affect the growth of the fetal small intestine (Meyer et al., 2010; Luther et al., 2007; Scholljegerdes et al., 2004). When evaluating nutritional restriction in sheep from the middle third of gestation, Reed et al. (2007) observed a decrease in small intestine mass and jejunal hypertrophy (protein: DNA), although another study did not

observe differences in the proliferation in jejunum (Neville et al., 2010). In these studies, lambs from sheep with nutritional restriction decreased the total microvascular volume of the jejunum along with the reduction of mRNA expression of the soluble guanylate cyclase 1 β3, a nitric oxide (NO) receptor that is involved in vasodilation and angiogenesis (Neville et al., 2010). Thus, alterations such as decrease in villus height and/or width (Avila et al., 1989; Cellini et al., 2004; D'Inca et al., 2010), crypt depth (Avila et al., 1989; Trahair et al., 1997) and mucosal size (Avila et al., 1989; Trahair et al., 1997; Wang et al., 2005) suggest that the decrease of formed mass can also be accompanied by a reduction of functional area.

However, the small intestine mass in relation to body weight was not always affected by IUGR (Avila et al., 1989; Wang et al., 2005). In assessing the effects of poor nutrition on ewes during the last three gestational weeks, Charlton and Johengen (1985) found that the fetal small intestine mass was not affected. Thus, they believe that longer periods of maternal nutritional restriction will affect the growth and development of the small intestine.

Duarte et al. (2013) carried out a study with cows that received feed restriction from the gestational day 47th. These authors observed that bovine fetuses presented greater vascularization and cell proliferation in the jejunum and greater relative size of the intestine and villus length, when expressed as a function of body weight. The authors comment that these facts could indicate a more efficient growth in order to compensate low nutrients supply.

Thus, we observed that the literature reports different effects on small intestine growth and development, depending on the level of maternal nutritional restriction and gestation time. Therefore, more studies are fundamental to understand the influence of maternal nutrition at specific moments of gestation on the cellular signaling mechanisms in the intestine and its consequences.

2.2.4 Effects of fetal programming on the mechanisms of digestion and absorption of nutrients at birth

Newborn ruminants are not functional ruminants. The establishment of this functionality occurs when these animals have access to solid feeds, allowing contact of the stomach compartments of the newborns, mainly of the rumen, with microorganisms (bacteria, fungi and protozoa).

After birth, lactose is the main source of energy in milk available to newborns mammals. When it is ingested, this carbohydrate reaches the intestinal lumen and undergoes action of lactase, an enzyme that cleaves lactose into glucose and galactose, molecules that are absorbed by the animal. The absorption of glucose and galactose occurs predominantly in the duodenum, jejunum, and to a lesser extent in the ileum, through the transmembrane transport performed by proteins (Shirazi-Beechey, 1995; Nguyen et al., 2012; Ran et al., 2016). These carrier proteins have distinct activities, but they are complementary. The sodium-dependent glucose transporter 1 (SGLT-1) is present in the apical membrane of the enterocyte and carries out the secondary active transport of these monosaccharides from the intestinal lumen to enterocytes, a process that requires ATP and is Na^+-dependent. This carrier has a Na^+ binding site, which at the time of binding allows the access of the glucose to the receptor. The transport of each glucose molecule requires two Na^+ molecules. By increasing the concentration of glucose in the intracellular space, the glucose transporter, GLUT2, transports the molecule to the bloodstream through the basolateral membrane, through of the facilitated diffusion process (Shirazi-Beechey, 1995; Kellett et al., 2008). GLUT2 has a high capacity to transport glucose, but has low affinity (Km for glucose > 50 mM). In addition to glucose, GLUT2 transports fructose and all other monosaccharides through the brush border of the enterocyte and the basolateral membrane (Liao et al., 2010).

The expression of SGLT1 is positively regulated by the glucose levels present in the intestinal lumen (Shirazi-Beechey et al., 1991), being relatively larger in the duodenum and jejunum (Nguyen et al., 2012; Ran et al., 2016). GLUT2 expression also occurs in the small intestine (Ran et al., 2016). These facts indicate that the duodenum and jejunum are critical regions for absorption of monosaccharides. In general, the uptake of monosaccharides into the intestinal epithelium through SGLT1 and GLUT2 may be influenced by several factors. Colostrum ingestion is one of them, since it stimulates the development and functionality of TGI, a fact observed in cattle (Buhler et al., 1998; Blum and Hammon, 2000; Blättler et al., 2001) and also in swine (Simmen et al., 1990; Odle et al., 1996; Xu et al., 2002). The components present in colostrum promote intestinal epithelial growth (Baumrucker et al., 1994; Blättler et al., 2001; Roffler et al., 2003) and promote a more effective absorption of glucose (Steinhoff-Wagner et al., 2011; Hammon et al., 2012). In addition, colostrum ingestion may stimulate the action of lactase in the small intestine of newborns (Tivey et al., 1994; Bird et al., 1996), which provides more glucose in the intestinal lumen to be absorbed and used by the

animal. In a study conducted by Qiu et al. (2005), IUGR rats showed increased activity of lactase enzymes and maltase in jejunum at birth, although this has not occurred in the postnatal period. The activity of the enzyme lactase was also higher in the jejunal brush border of newborn piglets, when there was maternal malnutrition throughout the gestation (Cao et al., 2014). These authors suggest that this would be a form of adaptation to compensate for the lack of nutritional supply during the prenatal life, causing IUGR animals to have higher digestive capacity.

Another factor that may influence the expression of glucose transporters in the small intestine is the glucagon-like peptide-2 (GLP-2). GLP-2 is a hormone composed of 33 amino acids, formed from cleavage of the proglucagon hormone (Dubé and Brubaker, 2007) and secreted by enteroendocrine L cells, in response to the presence of nutrients in the intestinal lumen (Rehfeld, 2004; Jang et al., 2007; Sato et al., 2013). According to Dube and Brubaker (2004), there may be a minimum calorie threshold necessary to induce GLP-2 secretion, regardless of the nutrient, as the infusion of low-level energy or low-calorie meals did not resulted in GLP secretion in many studies with humans and swine. In the literature, there are studies demonstrating that GLP-2 stimulates the expression of glucose transporters in enterocytes, including SGLT1 (Cheeseman, 1997; Ramsanahie et al., 2003; Cottrell et al., 2006) and GLUT2 (Cheeseman and O'neill, 1998; Au et al., 2002), and increases the expression of the digestive enzymes in the brush border, such as maltase-glucoamylase and sucrase-isomaltase (Petersen et al., 2001, 2002). In addition, GLP-2 has been shown to elevate intestinal blood flow and, consequently, to stimulate cellular proliferation of the small intestine through the release of nitric oxide from enteric neurons (Guan et al., 2003; Stephens et al., 2006; Dubé and Brubaker, 2007; Bremholm et al., 2009). These changes confer increased villus height (by increasing cell numbers) and crypt depth (Hartmann et al., 2000; Ramsanahie et al., 2004; Burrin et al., 2005, 2007). GLP-2 is also able to inhibit apoptosis of enterocytes (Tsai et al., 1997; Burrin et al., 2005, 2007). However, if the intake of enteric nutrients is deficient, the onset of the apoptotic process can be accelerated and lead to intestinal atrophy (Niinikoski et al., 2004; Ito et al., 2010; Dodge et al., 2012). The physiological activities of GLP-2 are mediated by a specific receptor (GLP-2R) coupled to protein G (Munroe et al., 1999). GLP-2R mRNA expression was observed throughout the gastrointestinal tract, including low levels in the rumen and omasus (Taylor-Edwards et al., 2010) and moderate levels in the duodenum, jejunum, ileum, cecum and rectum (Connor et al., 2010; Taylor-Edwards et al., 2010).

In addition to the effects on the uptake of nutrients, GLP-2 also protects the intestinal mucosa from inflammatory processes, promoting barrier function and healing after intestinal lesions (Lovshin and Drucker, 2000; Burrin et al., 2003; Drucker, 2005). Some studies have shown that GLP-2 increased protein expression of mRNA and tight junction proteins (TJ) in the jejunum of swine (Yu et al., 2014).

One of the major components of the epithelial barrier is the monolayer of juxtaposed columnar epithelial cells, which limits the luminal environment of the internal environment. Ions and molecules, including nutrients, can cross this intestinal epithelial monolayer through two pathways: the transcellular pathway and the paracellular pathway (Groschwitz and Hogan, 2009). The integrity of the paracellular pathway depends on the regulation of the TJ. This intercellular junction is a dynamic and adjustable structure whose permeability can change in response to various stimuli such as nutrients, the humoral or neuronal signaling or inflammatory mediators (Ulluwishewa et al., 2011; Collares-Buzato, 2013).

Under normal physiological conditions, the paracellular space forms a selective and semipermeable barrier to the ions and molecules, according to the respective load and size. This selectivity is provided by the TJ complex (Figure 4), which is composed of integral proteins, including occludin (the first protein of the complex to be identified) and members of the claudin family (27 isoforms are currently known). These proteins interact indirectly with the perijunctional ring of the actin cytoskeleton by cytoplasmic proteins such as zonula occludens - ZO-1, ZO-2 and ZO-3 (Gumbiner et al., 1991), cingulin (Gonzalez-Mariscal et al., 2003) and 7H6 antigen (Zhong et al., 1993). Integral membrane proteins (claudins and occludin) form sealing strands in the most apical region of the lateral cell membrane and the interaction of these strands is necessary to maintain the structural integrity of the barrier function of the intestinal epithelium (Van Itallie & Anderson 2014).

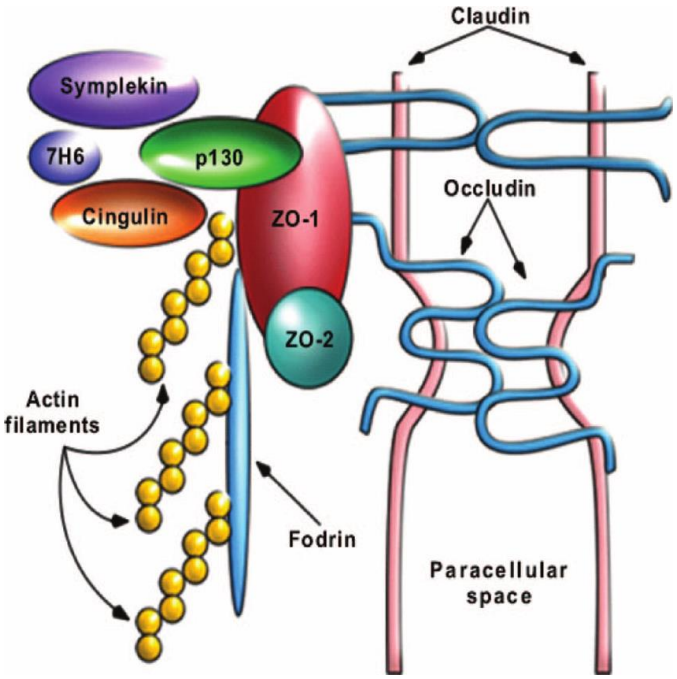

Figure 4. Molecular structure of the occlusion junction.
(Fasano, 2008)

Various pathologies affect the function of the intestinal barrier, including diabetes, inflammatory bowel diseases, celiac disease, food allergies, multiple sclerosis, and irritable bowel syndrome. However, the mechanisms that cause such deregulation are not yet fully elucidated (Rodrigues et al., 2016).

During gestation, any change in intestine development permanently affects the efficiency of the use of nutrients by the newborn (Trahair et al., 1997; Godfrey and Barker, 2000; Wu et al., 2006; Wang et al., 2008). The low nutrient availability to the fetus may compromise its growth, increasing the risk of intestinal disorders and consequently impairing the development of the function of the intestinal immune response (Han et al., 2013; Hu et al., 2015). Zhang et al. (2018) observed that IUGR lambs expressed less mRNA of Z0-1 and occludin in the ileum compared to lambs with birth weight considered normal. In addition, these authors reported that intestinal development, intestinal integrity, immune function and oxidative status were impaired in IUGR lambs.

Therefore, further studies are necessary on maternal feed restriction and their effects on the regulatory mechanisms of the intestinal epithelial barrier, digestive enzymes and nutrient transporters in the small intestine of the offspring, according to the feed restriction time. In

addition, further research involving strategies to improve the intestinal development of individuals with impaired prenatal growth and development is essential.

3 MATERIAL AND METHODS

3.1 Local

The experiment was conducted at the Goats Research Unit of Federal University of Viçosa, in Viçosa, Minas Gerais, following the animal welfare and management procedures, according to the Ethics Committee on the Use of Production Animals of the Federal University of Viçosa (protocol number 09/2017).

3.2 Animals, treatments and experimental design

Sixty nulliparous dairy goats, with initial mean (± standard deviation) body weight of 49 ± 13 kg and 19 ± 7 months, were submitted to estrus synchronization using prostaglandin (7 d interval between the first and second application) and artificial insemination protocol, as described by Fonseca et al. (2011). Semen from a single male was used, to avoid paternal effects. Day 0 (zero) of gestation was considered the day on which the animals were inseminated. The adaptation period was the 7 d after insemination. During the adaptation period, goats were confined in individual pens (3 m^2), receiving feed and water *ad libitum*. Gestation was confirmed by ultrasound (Aloka 500, 5 MHz probe, Aloka, Wallingford, CT, USA) 30 d after insemination and 14 goats, with 50 ± 13 kg and 19 ± 7 months, had gestation confirmed. At the 8^{th} day of gestation, these animals were randomly distributed into two groups with different feeding levels, where 8 of them were fed at the maintenance level (100% of total digestible nutrients, TDN, and crude protein, CP, according to National Research Council, 2007) until the 84^{th} day of gestation. From the 85^{th} day of gestation until parturition (term ~ 150 d), the dams were fed with 50% of TDN and CP (maintenance-restriction treatment, M-R). The other 6 goats were fed with 50% of TDN and CP until the 84^{th} d of gestation and received 100% of TDN and CP of National Research Council (2007) recommendations from the 85^{th} day of gestation to parturition date (restriction-maintenance treatment, R-M). Therefore, the treatments consisted of the same diet for both groups, with differences only in feeding and

gestational stages. The design of the application of treatments to goats during pregnancy is shown in Figure 5.

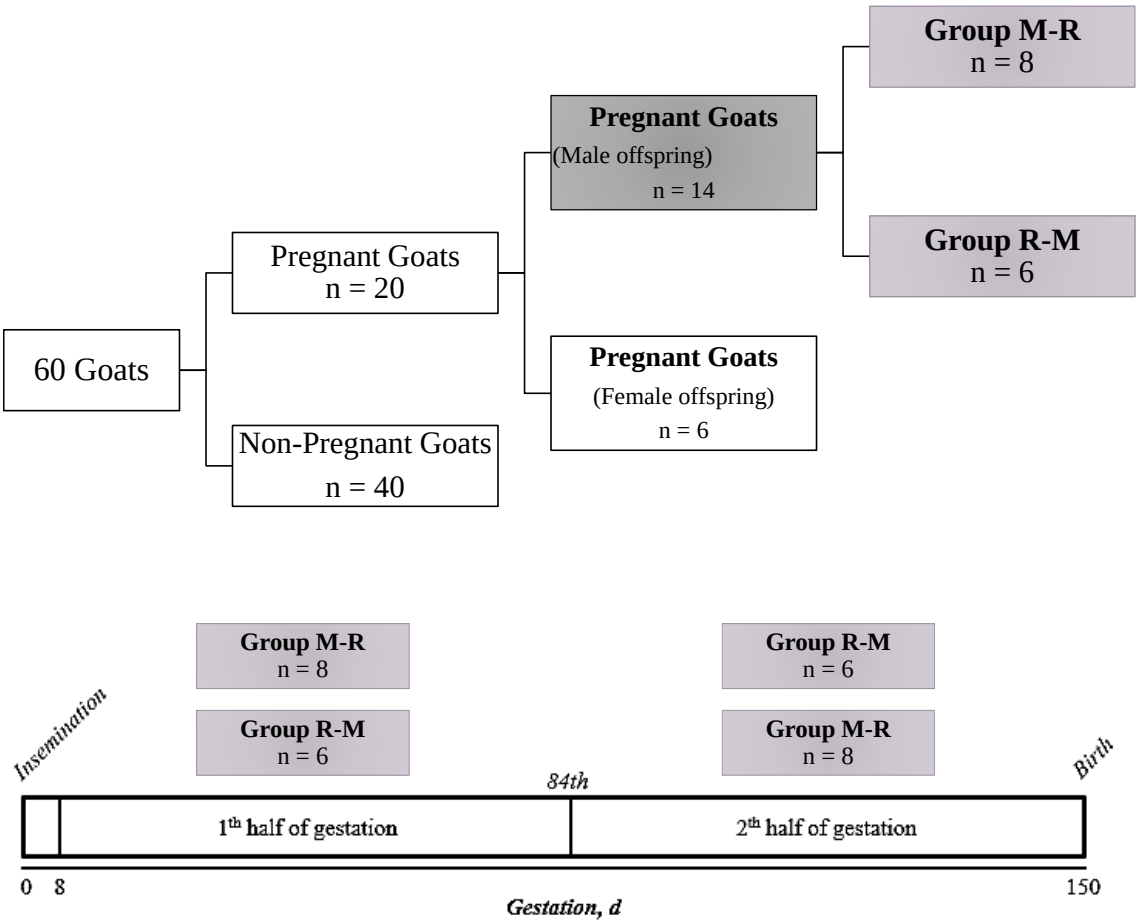

Figure 5. Design showing the application of experimental treatments (group M-R, feed restriction at the second half of gestation and group R-M, feed restriction at the first half of gestation) throughout gestation.

Both groups were fed once a day (at 7 a.m.) and every 7 d, the goats were weighed before feeding. The dry matter intake and daily supply of feed were adjusted weekly based on the body weight and week of gestation of the dams.

The experimental diets (Table 1) consisted of 111.6 g/kg of crude protein and 676 g/kg of total digestible nutrients on dry matter (DM) basis composed of corn silage (723 g/kg DM basis), soybean meal (96 g/kg DM basis), ground corn (165 g/kg DM basis), and mineral mixture (16 g/kg DM basis), considering dairy goats' nutritional requirements (National Research Council, 2007).

Table 1. Feeds and chemical composition of diet.

Item[1]	**Ingredients**	
(g × kg^{-1})	**Corn Silage**	**Concentrate**
DM†	257±6.93	845±0.04
OM‡	949±2.00	951±0.14
CP‡	71.2±1.55	145±3.07
EE‡	23.4±1.44	7.00±0.50
NDFap‡	542±9.21	147±26.2
NFC‡	313±9.94	652±29.7

[1]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)].
Concentrate composition: 96 g × kg^{-1} of soybean meal, 165 g × kg^{-1} of ground corn, 3 g × kg^{-1} of CaCO3 and 3 g × kg^{-1} of CaHPO4 (DM basis).
† g × kg^{-1} as feed
‡ g × kg^{-1} DM

3.3 Chemical analysis

Throughout the experimental period, the roughage and concentrate were sampled weekly. Daily, before feeding, leftovers were collected, weighed, sampled, identified and stored under -20 °C refrigeration, for further chemical analysis to evaluate the nutritional characteristics of the diet.

A five-day assay was performed, in the middle of each feeding period (in the 46 and 117 d of gestation) to evaluate the intake of dry matter (DMI), crude protein (CPI) and total digestible nutrient (TDNI). The diet provided, leftovers and total feces were collected and weighed every day during the assay. After the collection period, the daily samples of each animal were grouped into composite samples, one for each animal.

The roughage, concentrate, feces, and leftovers were oven-dried (55 °C/72 hours), grounded with 1 mm knife in a Wiley mill (Willye® TE-680), and analyzed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT- CA; Detmann et al., 2012) 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).

3.4 Performance evaluation of goats

At 8, 84, 85 d of gestation, goats were weighed in the morning and immediately before parturition to estimate total maternal ADG. The pregnant compound (PREG) approach (Gionbelli et al., 2015) was used to separate the maternal and gestational ADG. However, the bovine data from Gionbelli et al. (2015) was replaced by goat data considering the following equation as described by (Castagnino et al., 2015):

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

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

3.5 Newborn goat slaughter and collection of tissues samples

At birth, the male newborn goats were immediately separated from dams to avoid colostrum suction. This was done in order to avoid changes in blood glucose level. After collection of blood samples from offspring for subsequent commercial laboratory analysis by High-performance liquid chromatography (HPLC), the newborn goats were weighed and stunned by a non-penetrating captive bolt and exsanguinated. In the case of newborn twins (Treatment R-M, n = 4, Treatment M-R, n = 5), we chose to slaughter the heaviest. Liver, kidneys and gastrointestinal tract were carefully removed. The stomach complex were isolated from esophagus and the intestine at the pyloric valve and divided into reticulum-rumen omasum, abomasum and each component was emptied and weighed carefully. The large and small intestines were isolated, weighted and length was recorded separately similarly to that described by Duarte et al. (2013). The small intestine was sampled and properly stored for both further biomolecular and histological analysis. Kidneys, liver and mesentery were also were weighted.

3.6 Morphology of intestinal villi

Samples of jejunum, about 3 cm long, were collected carefully in the medial portion of the intestinal section and then fixed in fresh 10% (weight/volume) formalin in phosphate buffer (pH 7.4), were 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 cuts obtained from series was distended with distilled water, adhered in histological slide and dried in hot plate at 60 ºC. Then the sections were stained with toluidine blue for 10 seconds, 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 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.

3.7 Quantitative gene expression analysis

Total RNA was isolated from 0.03 g of each pulverized sample of small intestine (jejunum) of newborn goats using the SV Total RNA Isolation System kit (Promega Corporation, Madison, WI, USA), following the manufacturer's usage protocol. Subsequently, total RNA concentration was quantified by spectrophotometry, using the NanoVue spectrophotometer (GE Healthcare Life Sciences Inc.) and integrity 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 gene in the intestinal sample were synthesized and analyzed through the online programs OligoPerfect™ Designer (https://tools.thermofisher.com/content.cfm?pageid=9716) and OligoAnalyzer 3.1 (https://www.idtdna.com/calc/analyzer), with sequences obtained from GenBank (www.ncbi.nlm.nih.gov). In this research, β-actin was used as the reference gene for intestine of goats, as recommended by Zhang et al. (2013).

For the analysis of the quantitative gene expression by RT-qPCR, 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 were 2 minutes at 50 °C, 10 minutes at 95 °C followed by 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C, and ending with 15 seconds at 95 °C. For each reaction, 1.0 μL of cDNA, 0.3 μL of each primer (forward and reverse) and 5.0 μL of Master Mix SYBR Green were used for a last volume of 10.0 μL/96 wells MicroAmp Optical (Applied Biosystems, Foster City, CA, USA). The relative expression levels were calculated according to the method described by Pfaffl (2001) which is based on Ct values that are corrected for the amplification efficiency for each primer pair.

Table 2. List of primers used to quantify mRNA expression of genes in jejunum tissue using quantitative real-time PCR.

Gene	Gene abbreviation	NCBI access code	Primer	R^2	Efficiency
Glucagon like peptide 2 receptor	GLP2R	XM_005694224.3	FW: CGCTGGAAAACTCCACAGAT RV: GGCGTCCAACTTTTTGTTTG	0,970	1,121
Occludin	OCLN	NM_001082433.2	FW: AGCTGCCATTGACTTCACCT RV: CCTTTTTGAAAGCGTCTCCA	0,978	0,917
Solute carrier family 5 member 1	SLC5A1	HM060774.1	FW: CGTCATCTACTTCGTGGTGGT RV: GAAGAAGCCTCCAACAGTCC	0,998	0,980
Solute carrier family 2 member 2	SLC2A2	XM_005675321.3	FW: GCAGAGTTCCGAAAGAAGAGG RV: CAAAAAGCAGGTTATCTCTACATGG	0,996	1,016
Maltase-glucoamylase	MGAM	XM_018046851.1	FW: ATCACAAGATCCTGGGACGA RV: TCCGTTCCGAGTCATTTACC	0,996	1,005
Lactase	LCT	XM_018062350.1	FW: CTCCAGAACTGCCTCTCCAC RV: AAACCCAATGACGAGCACTT	0,991	1,094
Beta-actin	ACTB	JX046106.1	FW: GTCCACCTTCCAGCAGATGT RV: AGTCCGCCTAGAAGCATTTG	0,982	1,031

3.8 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 *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 (2 levels); T_j is the fixed effect of the j^{th} level of twins (2 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 dam 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 characteristics analyzed, effects in the model, with the exception of *D* and *T*, were removed when *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 gene expression data was not normal and it was transformed using the RANK procedure of SAS 9.2 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Least-squares means were separated using Fisher's least significant difference test.

Results were deemed significant when P-value ≤ 0.05 and tendency when 0.05 < *P*-value ≤ 0.10. All analyses were performed using SAS.

4 RESULTS

4.1 Maternal intake according to the period of feed restriction

In the first experimental period (8-84d gestation), there were differences in dry matter intake (DMI), crude protein intake (CPI) and total digestible nutrient intake (TDNI) between treatments. Goats that received feed restriction at this stage (treatment R-M) presented less intake ($P < 0.001$) compared to the goats of the treatment M-R. In the second experimental period (85d-parturition), goats that received feed restriction (treatment M-R) presented less DMI and CPI ($P < 0.001$) compared to the animals of the treatment R-M, but TDNI did not differ between the treatments ($P = 0.249$) (Table 3).

Table 3. Intake according to feed restriction of the dams during different stages (first or second half) of gestation.

Item[1]	**Treatment**		***P*-value**
(g/day)	**M-R**†	**R-M**‡	
	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

[1]Least square means ± standard errors of dry matter intake (DMI), crude protein (CPI) and total digestible nutrients intake (TDNI);
†M-R: maintenance-restriction treatment;
‡R-M: restriction-maintenance treatment.

4.2 Maternal performance according to the period of feed restriction

The Table 4 shows that the total average daily gain (ADG) of goats during the first half of gestation (8-84d gestation) and the second half (85d-parturition) differed ($P < 0.001$)

between treatments. However, no difference was observed in total ADG (*P = 0.411*) between treatments when considering the entire gestation period (8d - parturition).

The maternal tissues ADG was also different (*P < 0.001*) among treatments applied in the different stages of gestation (Table 4). However, when evaluating the maternal tissues ADG during the entire gestation, no differences between the treatments were observed (*P = 0.57*).

The gestation ADG did not differ between treatments during the first half of gestation (*P = 0.99*), second half of gestation (*P = 0.93*) and when evaluated throughout gestation (*P = 0.89*; Table 4).

Table 4. Performance according to feed restriction of the dams during different stages (first or second half) of gestation.

Item[1]	**Treatment**		***P*-value**
(g/day)	**M-R**†	**R-M**‡	
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

[1] Least square means ± standard errors of total average daily gain (ADG), maternal tissues ADG and gestation ADG of dams from the first (8 to 84d of gestation), the last (85d to parturition) experimental periods, and during the entire experimental period (8d to parturition);
†M-R: maintenance-restriction treatment;
‡R-M: restriction-maintenance treatment.

4.3 Body weight and blood glucose levels of offspring at birth

In Figure 6 are presented body weights and blood glucose levels of newborn goats from dams that were feed restricted in the first half of pregnancy (R-M) or in the second half of gestation (M-R). No differences were observed for body weight (*P = 0.46*), or blood glucose level (*P = 0.65*) of the offspring at birth from both treatments.

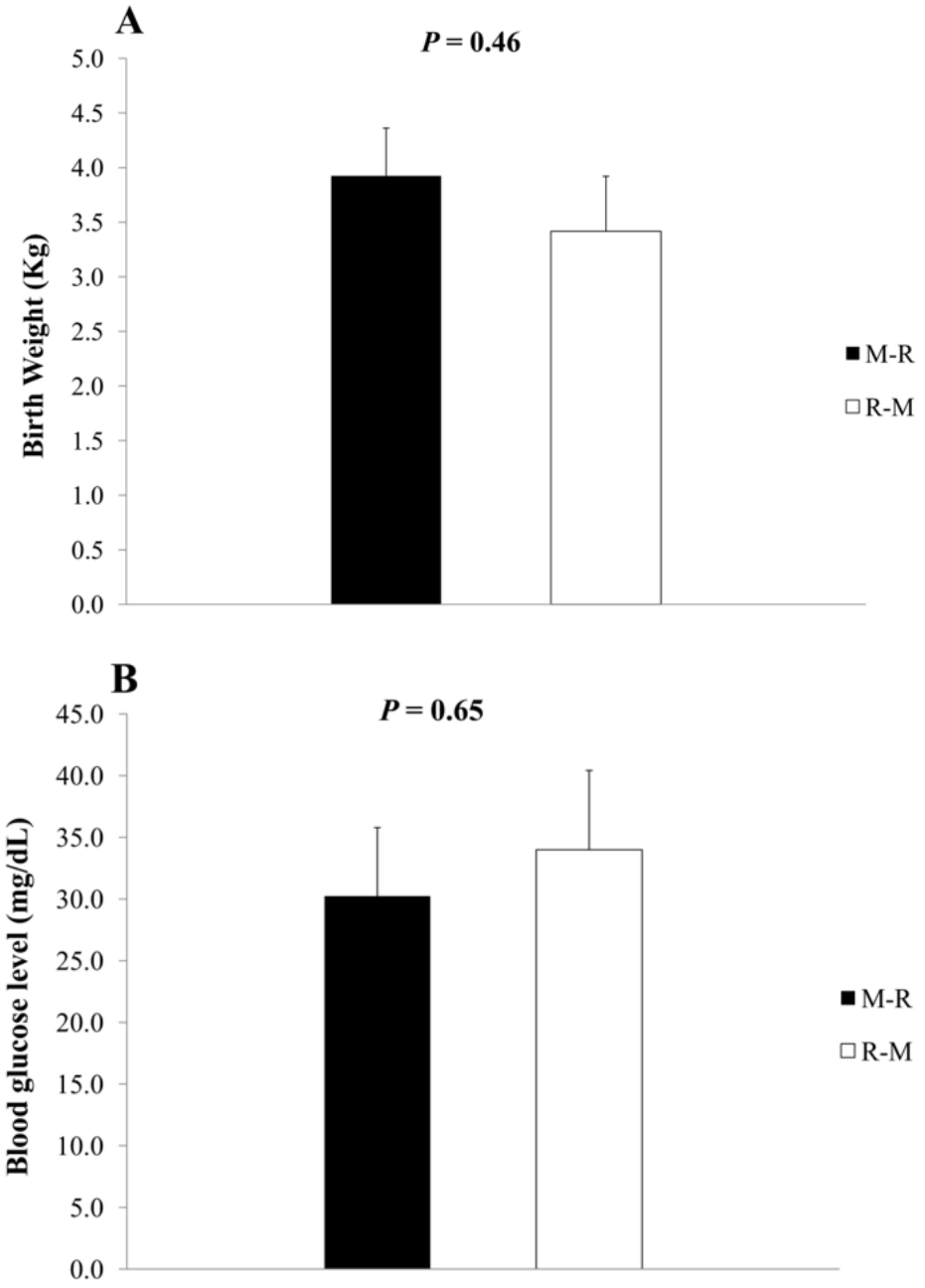

Figure 6. Average birth weight (A) of goat kids and blood glucose level (B).

4.4 Morphometric and morphology measurements of offspring

Morphometric and morphology data from slaughtered newborn at birth are presented in Table 5. Stomach compartments weight (reticulum-rumen, omasum and abomasum) did not differ between treatments (*P = 0.893*). However, when expressed as kg body weight there was tendency of greater weight of the reticulum-rumen and omasum (*P = 0.057*) of the M-R group compared to R-M group.

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. The lengths of small intestine and total intestine were also higher ($P \leq 0.05$) in the M-R group, however no differences were observed in the weight to length ratio of the small intestine and total intestine ($P > 0.46$) of offspring. In addition, maternal dietary treatments had no effect on large intestine measurements and organ weights of offspring ($P > 0.13$).

Table 5. Effects of maternal feed restriction at different gestational moments under gastrointestinal tract measurements, organ weights and jejunum morphology of offspring (least squares means ± SEM).

	Treatment		***P*-value**
Item	**M-R**[†]	**R-M**[‡]	
Gastrointestinal tract measurement			
Reticulum-rumen omasum (g)	18.9±0.456	18.8±0.529	0.893
Reticulum-rumen omasum (g × kg^{-1} BW)	5.43±0.0832	5.16±0.0964	0.057
Abomasum (g)	25.0±1.25	25.3±1.44	0.863
Abomasum (g × kg^{-1} BW)	6.70±0.341	6.69±0.393	0.986
Small intestine (g)	9.00±0.476	7.42±0.552	0.055
Small intestine (g × kg^{-1} BW)	2.46±0.130	2.00±0.150	0.038
Small intestine (cm)	93.3±5.14	75.5±5.94	0.043
Small intestine (g × cm^{-1})	0,0972±0,00448	0,0943±0,00518	0.680
Large intestine (g)	8.57± 0.682	7.45±0.790	0.311
Large intestine (g × kg^{-1} BW)	2.26±0.157	1.96±0.181	0.233

Large intestine (cm)	64.5±4.76	65.5±5.51	0.896
Large intestine (g × cm^{-1})	0.131±0.00801	0.111±0.00928	0.136
Total intestine (g)	17.6±0.957	14.9±1.11	0.095
Total intestine (g × kg^{-1} BW)	4.71±0.215	3.95±0.248	0.038
Total intestine (cm)	157±3.69	142±4.28	0.026
Total intestine (g × cm^{-1})	0.108±0.00446	0.102±0.00517	0.463
Organ weight			
Mesentery (g)	136±8.53	141±9.88	0.742
Mesentery (g × kg^{-1} BW)	36.3±2.32	36.5±2.68	0.964
Kidneys (g)	18.5±0.913	18.5±1.06	0.968
Kidneys (g × kg^{-1} BW)	5.02±0.206	5.06±0.238	0.891
Liver (g)	84.7±7.11	85.0±8.23	0.974
Liver (g × kg^{-1} BW)	22.5±1.44	22.8±1.66	0.898
Jejunum morphology			
Villi height (µm)	73.5±12.4	57.0±13.7	0.406
Crypt depth (µm)	98.1±9.99	130±11.6	0.081
Villus height to crypt depth ratio	0.704±0.0574	0.453±0.0629	0.016

†M-R: maintenance-restriction treatment
‡R-M: restriction-maintenance treatment

There was a tendency of greater crypt depth (*P = 0.081*) of newborn goats of the R-M group and consequently, these animals had lower villus height to crypt depth ratio (*P = 0.016*; Table 5; Figure 7). However, there was no difference in villus height (*P = 0.406;* Table 5).

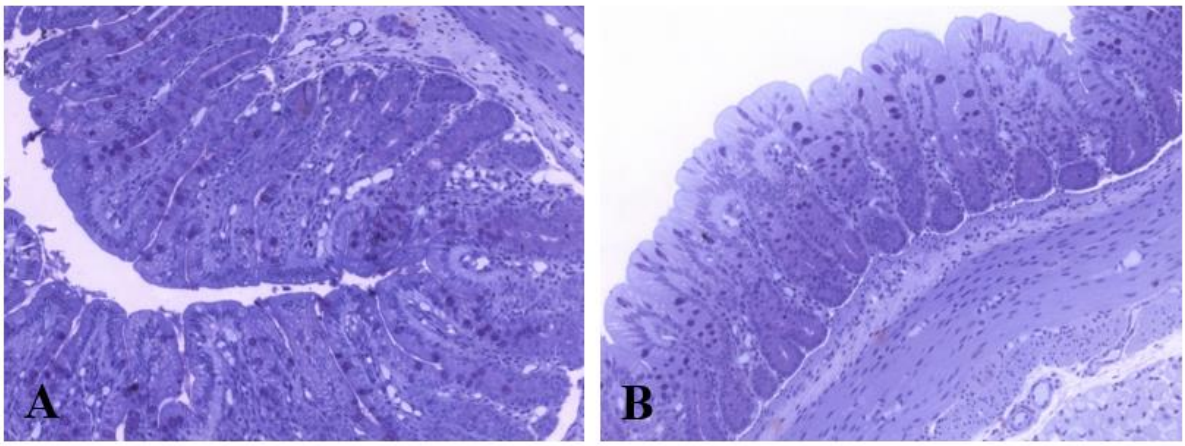

Figure 7. Photomicrographs of the jejunum of newborns of dams that received feed restriction in the first half of gestation (A) or in the second half of gestation (B).

4.5 Gene expression in jejunum of newborn goats

Gene expression in the jejunum of the newborns slaughtered at birth is presented in Figure 8. No effect of maternal feed treatment was observed in the mRNA expression of the MGAM and GLP-2R in the jejunum of the offspring ($P > 0.12$). 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 to animals born from twin gestation. Similarly, newborn goats from single gestated expressed more SLC2A2 mRNA ($P = 0.025$). In addition, a maternal feed restriction × number of fetuses interaction was observed for LCT mRNA expression ($P = 0.043$). Singletons of the R-M treatment expressed more LCT mRNA compared to the offspring of the M-R (single) and R-M (twin) groups, but no difference was observed when compared to the twins of the M-R group.

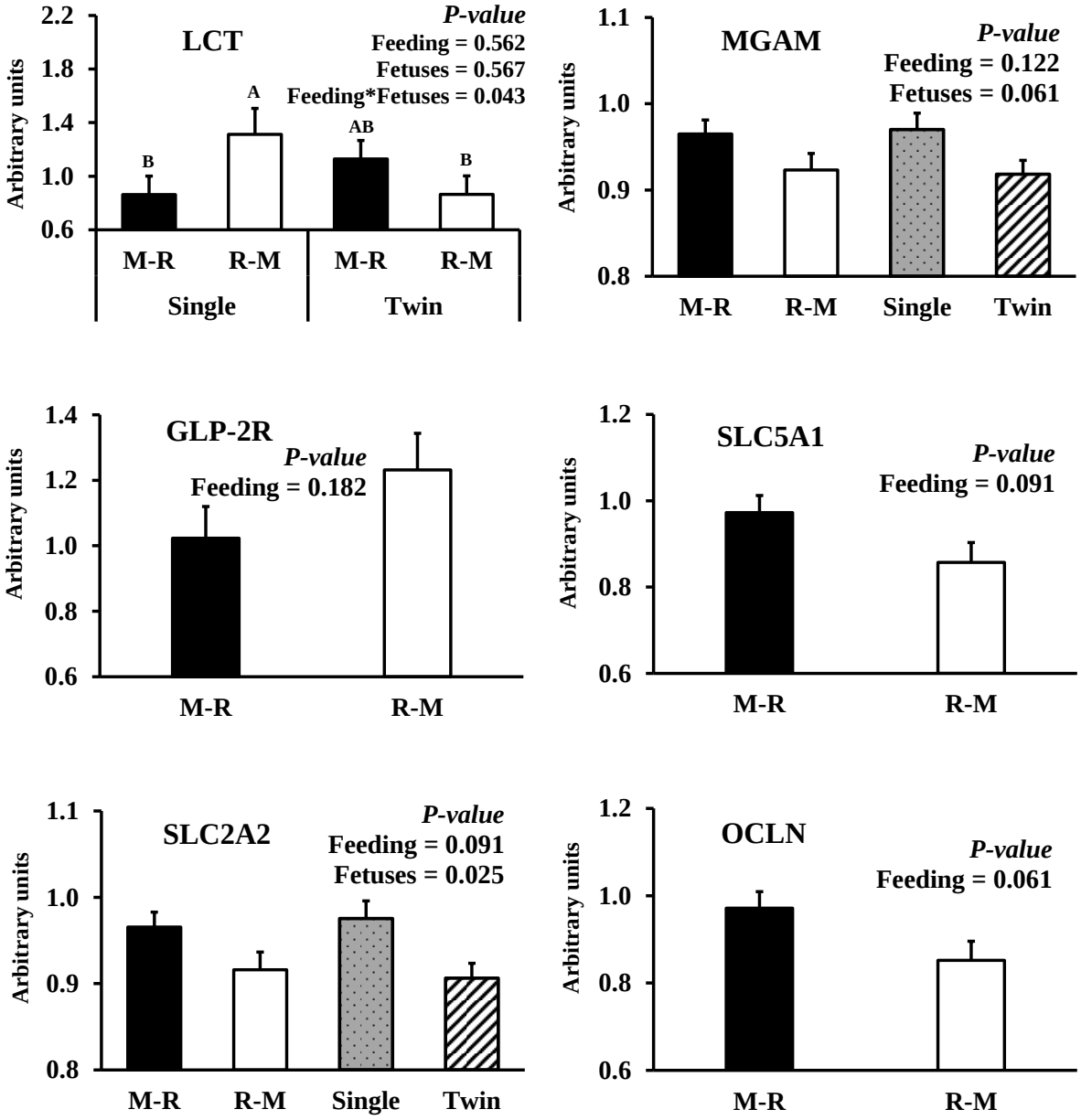

Figure 8. Gene expression in the jejunum according to maternal feeding level during gestation and number of fetuses.

5 DISCUSSION

The efficacy of maternal nutritional treatment applied at each stage of gestation was confirmed by the difference observed in total maternal ADG and especially in the ADG of maternal tissues. The results observed in the newborns demonstrate that this difference in maternal weight gain does not seem to influence fetal growth, since the gestation ADG and the

weight of the newborn did not differ between treatments. Other studies also observed the lack of difference in birth weight of offspring when there was a maternal restriction in the early to mid-gestation in cattle (Freetly et al., 2000, 2008; Meyer et al., 2010), or during the mid-to-late gestation in sheep (Carlson et al., 2008).

The maternal nutrition during pregnancy can affect the development of fetal (Funston and Summers, 2013). Although organogenesis occurs from the early to mid-gestation (Redmer et al., 2004; Fowden, 2006; Vonnahme et al., 2007; Caton et al., 2009; Meyer et al., 2010), the maternal feed restriction during the first half gestation had little or no effect on gastrointestinal tract compartments and other organs of the newborns in the present study, except for the characteristics of the small intestine. The lack of difference in most visceral organs corroborates with results obtained in other studies with sheep (Osgerby et al., 2002) and cows (Molle et al., 2004; Meyer et al., 2010). A possible way to compensate for the low nutrient supply would be to increase the total number of caruncles in placenta of sheep with nutritional restriction (Clarke et al., 1998). In addition, Meyer et al. (2010) suggested that the growth rate of fetal visceral organs may be accelerated due to realimentation of dams after the restraint period. These authors also comment that the accelerated or compensatory fetal organ growth rate may occur due to the increase of nutrient use efficiency by the dam or the fetus, or both, after the maternal restriction. Cows receiving restricted feed may require fewer nutrients for maintenance, which may allow more nutrients to be divided into fetal growth (Meyer et al., 2010). In contrast, the fetus may also alter blood flow to different organs during development to try to rescue important tissues (Nathanielsz, 2006).

Visceral organs, including stomach complex and small intestine, are particularly sensitive to nutrient restriction (Reed et al., 2007). In the absence of adequate nutrition, the fetus sacrifices tissues, such as intestine and muscle, for the prioritization of brain growth (Desai et al., 2005; Fall, 2009). According to 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 presented a greater mass of these organs per unit of body weight at 125 d of gestation. The results observed in the present study for reticulum-rumen omasum, small intestine, total intestine, confirm that the mass proportional to the fetus or the absolute mass of these compartments are more affected when the restriction occurs in the first half of gestation. This is in agreement with another study, in which the authors observed a reduction in the mass of the small intestine and the total gastrointestinal tract in ovine fetuses with a nutritional

restriction in the first half of gestation (Trahair et al., 1997). In the present study, the lengths of the small intestine and total intestine were also affected by maternal feed restriction from the 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). In addition, the length and morphological components of the intestine are important factors for intestinal development and consequently, nutrient absorption (Trahair et al., 1997).

The morphological results obtained in this study demonstrated that there are differences in crypt depth and villus height to crypt depth ratio of newborns, depending on the stage of pregnancy that the restriction occurs. The tendency of increased proliferation of the jejunal crypt in R-M animals corroborates with another study conducted in cattle with nutritional restriction from the early to the 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; Rodrigues et al., 2016). In addition, villus height is inversely associated with the proliferation rates of epithelial cells (Blättler et al., 2001; Sauter et al., 2004). Thus, it was hypothesized that the lack of difference in villus height and the greater crypt depth of offspring R-M indicate possible adaptive mechanisms that these newborns developed to compensate for the reduction of intestinal length. In addition, the greater crypt depth observed in these animals may be considered a potential for increased nutrient uptake in later stages. However, not only measurements of intestinal length and morphological characteristics should be taken into account when analyzing the development and maturation of intestinal function. The objective of including analyzes of gene expression in this study is to evaluate the functional capacity of the intestines of restricted newborns, in relation to digestion, absorption and intestinal permeability.

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 (Drozdowski and Thomson, 2006). Any change in the activity of these enzymes can affect the absorption of the 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., 1991b), while maltase and sucrose activities increase at the end of this period (Kidder and Manners, 1980; Kelly et al., 1991b). 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 indicate a faster maturation of the jejunal epithelium (Tian et al., 2018).

To evaluate the mechanisms involved in the regulation of enzymatic activity and intestinal development, we investigated the gene expression of the enzymes lactase and maltase in the present study. Maternal feed restriction alone had no effect on the mRNA expression of the enzymes evaluated. However, an interaction between maternal dietary treatment and fetal numbers (single or twin) was found in the gene expression of lactase. The results indicate that the singletons of the R-M treatment expressed more LCT mRNA compared to the offspring of the M-R (single) and R-M (twin) groups, but no difference was observed when compared to the twins of the M-R group. It is believed that the greater mRNA expression of lactase in the singletons of the R-M group was due to maternal realimentation 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 realimentation, simple gestation also favored gene expression of this enzyme, since it was previously verified that the competition for limited nutrients between the two fetuses of a same gestation can 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, researches in prolific species, including rats and pigs, has already been performed to evaluate the activity of digestive enzymes. In a study conducted with IUGR rats, it was possible to observe a higher activity of the enzyme lactase and maltase in the jejunum of these animals at birth compared to 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 to present LCT mRNA expression similar to the other offspring in the present study. In addition, the increased competition for nutrients between the fetuses of twin gestations discussed earlier, may also justify the tendency of lower expression of MGAM mRNA in these animals compared to singletons.

In an attempt to better understand how maternal feed restriction may influence intestinal growth and development, we also analyze the expression of the gene encoding the receptor that binds to GLP-2. GLP-2 is an endogenous regulatory peptide that possesses potent trophic activity on the intestinal mucosa (Brubaker et al., 1997; Tsai et al., 1997; Lovshin et al., 2000; Yusta et al., 2000; Drucker, 2001). In addition, GLP-2 is considered a modulator of the activity and expression of intestinal nutrient transporters, including SGLT1 (Cheeseman, 1997; Ramsanahie et al., 2003; Cottrell et al., 2006) and GLUT2 (Cheeseman and O'neill, 1998; Au et al., 2002). SGLT1 and GLUT2 are encoded from the Solute carrier family 5 member 1 (SLC5A1) and Solute carrier family 2 member 2 (SLC2A2) genes, respectively. In newborn piglets, maternal nutritional restriction did not affect GLP-2 receptor mRNA expression and SLC5A1 and SLC2A2 transporters when compared to 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 indicate that adequate nutritional intake during the first half of gestation seems to be indispensable to increase the expression of these intestinal transporters at birth. Moreover, the fact that newborn twins present lower expression of SLC2A2 mRNA, independent of maternal feeding, indicates that these animals are 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 passive transfer of Ig through maternal colostrum to protect it against infections until your immune system is established. In the first 24 hours after birth, the newborn small intestine is permeable to absorption of intact macromolecules, including Ig and other proteins. Hammer et al. (2011) reported that lambs whose dams had nutritional restriction during the 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 seem to be responsible for the formation of a barrier for macromolecules (Rodrigues et al., 2016). Similarly, Zhang et al. (2018) found that IUGR lambs expressed less mRNA Z0-1 and occludin in the ileum compared to 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 a shorter intestine. However, increased permeability and lower selectivity may predispose to increased absorption of bacteria and harmful substances into the body through the intestinal mucosa. Especially when it comes to newborn animals due to the immature immune system that can cause inflammatory processes. In addition, more studies are needed with this type of approach to evaluate the performance of these animals until adulthood.

6 CONCLUSION

Severe feed restriction of the dams at distinct stages of gestation alters differently the growth and development of the small intestine of the offspring. Even without changes in body weight and blood glucose levels of newborns, the changes in morphometry, morphology and gene expression in the jejunum of offspring indicate that restriction in the first half of pregnancy may affect the health and performance of offspring throughout life.

REFERENCES

Au, A., A. Gupta, P. Schembri, and C. I. Cheeseman. 2002. Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. 254:247–254.

Avila, C. G., R. Harding, S. Rees, and P. M. Robinson. 1989. Small intestinal development in growth-retarded fetal sheep. J. Pediatr. Gastroenterol. Nutr. 8:507–515.

Barker, D. 1997. Fetal undernutrition and disease in later life. Rev. Reprod. 2:105–112. doi:10.1530/ror.0.0020105. Available from: http://www.reproduction-online.org/lookup/doi/10.1530/ror.0.0020105

Barker, D. J. ., K. . Godfrey, P. . Gluckman, J. . Harding, J. . Owens, and J. . Robinson. 1993. Fetal nutrition and cardiovascular disease in adult life. Lancet. 341:938–941. doi:10.1016/0140-6736(93)91224-A.

Barrell, G. K., S. M. Moenter, A. Caraty, and F. J. Karsch. 1992. Seasonal changes of gonadotropin-releasing hormone secretion in the ewe. Biol. Reprod. 46:1130–5. doi:10.1095/biolreprod46.6.1130. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1391310

Bauman, D. E., and W. Bruce Currie. 1980. Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis. J. Dairy Sci. 63:1514–1529. doi:10.3168/jds.S0022-0302(80)83111-0. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022030280831110

Baumrucker, C. R., D. L. Hadsell, and J. W. Blum. 1994. Effects of dietary insulin-like growth factor I on growth and insulin-like growth factor receptors in neonatal calf intestine. J. Anim. Sci. 72:428–433.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804–2819. doi:10.2527/1995.7392804x.

Bell, A. W., and R. A. Ehrhardt. 2000. Regulation of macronutrient partitioning between maternal and conceptus tissues in the pregnant ruminant. Rumin. Physiol. Dig. Metab. growth Reprod. PB Cronjé) pp. 275–293.

Bell, A. W., C. L. Ferrell, and H. C. Freetly. 1993. Pregnancy and fetal metabolism. Quant. Asp. Rumin. Dig. Metab. 405–431.

Bell, A. W., P. L. Greenwood, and R. A. Ehrhardt. 2005. Regulation of metabolism and growth during prenatal life. Biol. Grow. Anim. 3:3–34.

Bird, A. R., W. J. J. Croom, Y. K. Fan, B. L. Black, B. W. McBride, and I. L. Taylor. 1996. Peptide regulation of intestinal glucose absorption. J. Anim. Sci. 74:2523–2540.

Bittrich, S., C. Philipona, H. M. Hammon, and V. Rome. 2004. Preterm as Compared with Full-Term Neonatal Calves Are Characterized by Morphological and Functional Immaturity of the

Small Intestine *. 1786–1795. doi:10.3168/jds.S0022-0302(04)73334-2.

Blättler, U., H. Hammon, C. Morel, C. Philipona, A. Rauprich, V. Rome, I. Le Huërou-Luron, P. Guilloteau, and J. W. Blum. 2001. Feeding Colostrum, Its Composition and Feeding Duration Variably Modify Proliferation and Morphology of the Intestine and Digestive Enzyme Activities of Neonatal Calves.

Blum, J. W. 2006. Nutritional physiology of neonatal calves. J. Anim. Physiol. Anim. Nutr. (Berl). 90:1–11. doi:10.1111/j.1439-0396.2005.00614.x.

Blum, J. W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livest. Prod. Sci. 66:151–159. doi:10.1016/S0301-6226(00)00222-0.

Bremholm, L., M. Hornum, B. M. Henriksen, S. Larsen, and J. J. Holst. 2009. Glucagon-like peptide-2 increases mesenteric blood flow in humans. Scand. J. Gastroenterol. 44:314–319.

Brubaker, P. L., A. Izzo, M. Hill, and D. J. Drucker. 1997. Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. Am. J. Physiol. Metab. 272:E1050–E1058.

Buhler, C., H. Hammon, G. L. Rossi, and J. W. Blum. 1998. Small intestinal morphology in eight-day-old calves fed colostrum for different durations or only milk replacer and treated with long-R3-insulin-like growth factor I and growth hormone. J. Anim. Sci. 76:758–765.

Burrin, D. G., B. Stoll, and X. Guan. 2003. Glucagon-like peptide 2 function in domestic animals. Domest. Anim. Endocrinol. 24:103–122.

Burrin, D. G., B. Stoll, X. Guan, L. Cui, X. Chang, and D. Hadsell. 2007. GLP-2 rapidly activates divergent intracellular signaling pathways involved in intestinal cell survival and proliferation in neonatal piglets. Am. J. Physiol. Metab.

Burrin, D. G., B. Stoll, X. Guan, L. Cui, X. Chang, and J. J. Holst. 2005. Glucagon-like peptide 2 dose-dependently activates intestinal cell survival and proliferation in neonatal piglets. Endocrinology. 146:22–32.

Cao, M., L. Che, J. Wang, M. Yang, G. Su, Z. Fang, Y. Lin, S. Xu, and D. Wu. 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. doi:10.1016/j.nut.2014.04.016.

Carlson, D. B., J. J. Reed, P. P. Borowicz, J. B. Taylor, L. P. Reynolds, T. L. Neville, D. A. Redmer, K. A. Vonnahme, and J. S. Caton. 2008. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes 1. 669–680. doi:10.2527/jas.2007-0837.

Castagnino D de S, Härter CJ, Rivera AR, Lima LD de, Silva HG de O, Biagioli B, et al. Changes in maternal body composition and metabolism of dairy goats during pregnancy. Rev Bras Zootec 2015;44:92–102. doi:10.1590/S1806-92902015000300003..

Caton, J. S., J. J. Reed, R. P. Aitken, J. S. Milne, P. P. Borowicz, L. P. Reynolds, D. A. Redmer, and J. M. Wallace. 2009. Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J. Anim. Sci. 87:222–235. doi:10.2527/jas.2008-1043.

Cellini, C., J. Xu, A. Arriaga, and T. L. Buchmiller-Crair. 2004. Effect of epidermal growth factor infusion on fetal rabbit intrauterine growth retardation and small intestinal development. J. Pediatr. Surg. 39:891–897. doi:10.1016/j.jpedsurg.2004.02.008.

Charlton, V., and M. Johengen. 1985. Effects of intrauterine nutritional supplementation on fetal growth retardation. Biol. Neonate. 48:125–142.

Cheeseman, C. I. 1997. Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo. Am. J. Physiol. 273:R1965-71.

Cheeseman, C. I., and D. O'neill. 1998. Basolateral D-glucose transport activity along the crypt-villus axis in rat jejunum and upregulation induced by gastric inhibitory peptide and glucagon-like peptide-2. Exp. Physiol. 83:605–616.

Cheng, H., and C. P. Leblond. 1974. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine V. Unitarian theory of the origin of the four epithelial cell types. Am. J. Anat. 141:537–561.

Collares-Buzato, C. B. 2013. Junções Celulares. H. F. Carvalho S. M. Recco-Pimentel (Eds.), A Célula,. 141–166.

Connor, E. E., R. L. B. Vi, A. V Capuco, S. E. Ellis, and K. S. Sciabica. 2010. Characterization of glucagon-like peptide 2 pathway member expression in bovine gastrointestinal tract. J. Dairy Sci. 93:5167–5178. doi:10.3168/jds.2010-3205. Available from: http://dx.doi.org/10.3168/jds.2010-3205

Cottrell, J. J., B. Stoll, R. K. Buddington, J. E. Stephens, L. Cui, X. Chang, and D. G. Burrin. 2006. Glucagon-like peptide-2 protects against TPN-induced intestinal hexose malabsorption in enterally refed piglets. Am. J. Physiol. Gastrointest. Liver Physiol. 290:G293–G300. doi:10.1152/ajpgi.00275.2005.

Council, N. R. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. The National Academies Press, Washington, DC. Available from: https://www.nap.edu/catalog/11654/nutrient-requirements-of-small-ruminants-sheep-goats-cervids-and-new

Crosnier, C., D. Stamataki, and J. Lewis. 2006. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat. Rev. Genet. 7:349. Available from: https://doi.org/10.1038/nrg1840

D'Inca, R., M. Kloareg, C. Gras-Le Guen, and I. Le Huerou-Luron. 2010. Intrauterine Growth Restriction Modifies the Developmental Pattern of Intestinal Structure, Transcriptomic Profile,

and Bacterial Colonization in Neonatal Pigs. J. Nutr. 140:925–931. doi:10.3945/jn.109.116822. Available from: http://jn.nutrition.org/cgi/doi/10.3945/jn.109.116822

Desai, M., D. Gayle, J. Babu, and M. G. Ross. 2005. Permanent reduction in heart and kidney organ growth in offspring of undernourished rat dams. Am. J. Obstet. Gynecol. 193:1224–1232.

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

Dodge, M. E., R. F. Bertolo, and J. A. Brunton. 2012. Enteral feeding induces early intestinal adaptation in a parenterally fed neonatal piglet model of short bowel syndrome. J. Parenter. Enter. Nutr. 36:205–212. doi:10.1177/0148607111417447.

Drozdowski, L., and A. B. R. Thomson. 2006. Intestinal sugar transport. 12:1657–1670.

Drucker, D. J. 2001. Glucagon-like peptide 2. J. Clin. Endocrinol. Metab. 86:1759–1764.

Drucker, D. J. 2005. Biologic actions and therapeutic potential of the proglucagon-derived peptides. Nat. Rev. Endocrinol. 1:22.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88. doi:10.2527/jas.2009-2311.

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

Dube, P. E., and P. L. Brubaker. 2004. Nutrient, neural and endocrine control of glucagon-like peptide secretion. Horm. Metab. Res. 36:755–760.
Dubé, P. E., and P. L. Brubaker. 2007. Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. Am. J. Physiol. Metab.

Fall, C. 2009. Maternal nutrition: effects on health in the next generation. Indian J. Med. Res. 130.

Fasano, A. 2008. Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall. Am. J. Pathol. 173:1243–1252.

Fonseca, J. F. Da, R. D. C. Cruz, P. H. N. Pinto, and O. Facó. 2011. On line Manual de Indução e Sincronização de Estro e Ovulação em Ovinos e. 59. Available from: www.cnpc.embrapa.br

Fowden, A. L. 2006. Intrauterine Programming of Physiological Systems: Causes and Consequences. Physiology. 21:29–37. doi:10.1152/physiol.00050.2005. Available from: http://physiologyonline.physiology.org/cgi/doi/10.1152/physiol.00050.2005

Freetly, H. C., C. L. Ferrell, T. G. Jenkins, H. C. Freetly, C. L. Ferrell, and T. G. Jenkins. 2000. Timing of realimentation of mature cows that were feed-restricted during pregnancy influences calf birth weights and growth rates The online version of this article , along with updated information and services , is located on the World Wide Web at : Timi. 2790–2796.

Freetly, H. C., J. A. Nienaber, and T. Brown-Brandl. 2008. Partitioning of energy in pregnant beef cows during nutritionally induced body weight fluctuation. J. Anim. Sci. 86:370–377. doi:10.2527/jas.2007-0250.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: implications for beef cattle production. J. Anim. Sci. 88. doi:10.2527/jas.2009-2351.

Funston, R. N., and A. F. Summers. 2013. Epigenetics: Setting up lifetime production of beef cows by managing nutrition. Annu. Rev. Anim. Biosci. 1:339–363.

Gionbelli, M. P., M. S. Duarte, S. C. V. Filho, E. Detmann, M. L. Chizzotti, F. C. Rodrigues, D. Zanetti, T. R. S. Gionbelli, and M. G. Machado. 2015. Achieving body weight adjustments for feeding status and pregnant or non-pregnant condition in beef cows. PLoS One. 10. doi:10.1371/journal.pone.0112111.

Gionbelli, T. R. S., P. P. Rotta, C. M. Veloso, S. C. Valadares Filho, B. C. Carvalho, M. I. Marcondes, M. F. L. Ferreira, J. V. F. Souza, J. S. A. A. Santos, L. C. Lacerda, M. S. Duarte, and M. P. Gionbelli. 2016. Intestinal development of bovine foetuses during gestation is affected by foetal sex and maternal nutrition. J. Anim. Physiol. Anim. Nutr. (Berl). 101:493–501. doi:10.1111/jpn.12572.

Godfrey, K. M., and D. J. Barker. 2000. Fetal nutrition and adult disease. [Review] [85 refs]. Am. J. Clin. Nutr. 71:1344S–1352S. doi:10.1111/j.1365-277X.2005.00612.x.

Gonzalez-Mariscal, L., A. Betanzos, P. Nava, and B. E. Jaramillo. 2003. Tight junction proteins. Prog. Biophys. Mol. Biol. 81:1–44.

Groschwitz, K. R., and S. P. Hogan. 2009. Intestinal barrier function: molecular regulation and disease pathogenesis. J. Allergy Clin. Immunol. 124:3–20.

Gruenwald, P. 1963. Normal and abnormal expansion of the lungs of newborn infants obtained at autopsy III. The pattern of aeration as affected by gestational and postnatal age. Anat. Rec. 146:337–351. doi:10.1002/ar.1091460407. Available from: http:https://doi.org/10.1002/ar.1091460407

Guan, X., B. Stoll, X. Lu, K. A. Tappenden, J. J. Holst, B. Hartmann, and D. G. Burrin. 2003. GLP-2-mediated up-regulation of intestinal blood flow and glucose uptake is nitric oxide-dependent in TPN-fed piglets. Gastroenterology. 125:136–147. doi:10.1016/S0016-5085(03)00667-X.

Gumbiner, B., T. Lowenkopf, and D. Apatira. 1991. Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. Proc. Natl. Acad. Sci. 88:3460–3464.

Hales, C. N., and D. J. P. Barker. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Int. J. Epidemiol. 42:1215–1222. doi:10.1093/ije/dyt133.

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

Hammon, H. M., J. Steinhoff-Wagner, U. Schönhusen, C. C. Metges, and J. W. Blum. 2012. Energy metabolism in the newborn farm animal with emphasis on the calf: Endocrine changes and responses to milk-born and systemic hormones. Domest. Anim. Endocrinol. 43:171–185. doi:10.1016/j.domaniend.2012.02.005. Available from: http://dx.doi.org/10.1016/j.domaniend.2012.02.005

HAMMOND, J. 1947. ANIMAL BREEDING IN RELATION TO NUTRITION AND ENVIRONMENTAL CONDITIONS. Biol. Rev. 22:195–213. doi:10.1111/j.1469-185X.1947.tb00330.x. Available from: http:https://doi.org/10.1111/j.1469-185X.1947.tb00330.x

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

Harding, J. E., C. T. Jones, and J. S. Robinson. 1985. Studies on experimental growth retardation in sheep. The effects of a small placenta in restricting transport to and growth of the fetus. J. Dev. Physiol. 7:427–442.

Hartmann, B., M. B. Harr, P. B. Jeppesen, M. Wojdemann, C. F. Deacon, P. B. Mortensen, and J. J. Holst. 2000. In vivo and in vitro degradation of glucagon-like peptide-2 in humans. J Clin Endocrinol Metab. 85:2884–2888. doi:10.1210/jcem.85.8.6717.

Henning, S. J. 1985. Ontogeny of Enzymes in the Small IntestineHenning, S. J. 1985. Ontogeny of Enzymes in the Small Intestine. Annu. Rev. Physiol. 47:231–245. doi:10.1146/annurev.ph.47.030185.001311. Available from: https://doi.org/10.1146/annurev.ph.47.030185.001311. Annu. Rev. Physiol. 47:231–245. doi:10.1146/annurev.ph.47.030185.001311. Available from: https://doi.org/10.1146/annurev.ph.47.030185.001311

Hu, L., Y. Liu, C. Yan, X. Peng, Q. Xu, Y. Xuan, F. Han, G. Tian, Z. Fang, and Y. Lin. 2015. Postnatal nutritional restriction affects growth and immune function of piglets with intra-uterine growth restriction. Br. J. Nutr. 114:53–62.

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

http://dx.doi.org/10.1016/j.livsci.2015.11.003

Ito, J., H. Uchida, T. Yokote, K. Ohtake, and J. Kobayashi. 2010. Fasting-induced intestinal apoptosis is mediated by inducible nitric oxide synthase and interferon-{gamma} in rat. Am. J. Physiol. Gastrointest. liver Physiol. 298:G916–G926. doi:10.1152/ajpgi.00429.2009. Available from: papers2://publication/doi/10.1152/ajpgi.00429.2009

Jang, H.-J., Z. Kokrashvili, M. J. Theodorakis, O. D. Carlson, B.-J. Kim, J. Zhou, H. H. Kim, X. Xu, S. L. Chan, M. Juhaszova, M. Bernier, B. Mosinger, R. F. Margolskee, and J. M. Egan. 2007. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proc. Natl. Acad. Sci. 104:15069–15074. doi:10.1073/pnas.0706890104. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0706890104

Jiang, H., and B. A. Edgar. 2012. Intestinal stem cell function in Drosophila and mice. Curr. Opin. Genet. Dev. 22:354–360. doi:https://doi.org/10.1016/j.gde.2012.04.002. Available from: http://www.sciencedirect.com/science/article/pii/S0959437X12000469

Johnson Johnson, KA and Baldwin, RL, D. E. 1990. Changes in liver and gastrointestinal tract energy demands in reponse to physiological workloadin ruminants. J. Nutr. 120:649–655.

Kellett, G. L., E. Brot-laroche, O. J. Mace, and A. Leturque. 2008. Sugar Absorption in the Intestine : The Role of GLUT2. doi:10.1146/annurev.nutr.28.061807.155518.

Kelly, D., T. P. King, M. McFadyen, and A. J. Travis. 1991a. Effect of lactation on the decline of brush border lactase activity in neonatal pigs. Gut. 32:386–392.

Kelly, D., J. A. Smyth, and K. J. McCracken. 1991b. 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. Br. J. Nutr. 65:169–180.

Kenyon, P. R., and H. T. Blair. 2014. Foetal programming in sheep–effects on production. Small Rumin. Res. 118:16–30.

Kidder, D. E., and M. J. Manners. 1980. The level and distribution of carbohydrases in the small intestine mucosa of pigs from 3 weeks of age to maturity. Br. J. Nutr. 43:141–153.

Kotsampasi, B., S. Chadio, G. Papadomichelakis, S. Deligeorgis, D. Kalogiannis, I. Menegatos, and G. Zervas. 2009. Effects of maternal undernutrition on the hypothalamic–pituitary–gonadal axis function in female sheep offspring. Reprod. Domest. Anim. 44:677–684.

Liao, S. F., D. L. Harmon, E. S. Vanzant, K. R. McLeod, J. A. Boling, and J. C. Matthews. 2010. The small intestinal epithelia of beef steers differentially express sugar transporter messenger ribonucleic acid in response to abomasal versus ruminal infusion of starch hydrolysate. J. Anim. Sci. 88:306–314.

Long, N. M., M. J. Nijland, P. W. Nathanielsz, and S. P. Ford. 2010. The effect of early to mid-gestational nutrient restriction on female offspring fertility and hypothalamic-pituitary-adrenal

axis response to stress. J. Anim. Sci. 88:2029–2037.

Lovshin, J., and D. J. Drucker. 2000. New frontiers in the biology of GLP-2. Regul. Pept. 90:27–32.

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

Lunn, P. G., C. A. Northrop-Clewes, and R. M. Downes. 1991. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. Lancet. 338:907–910. doi:10.1016/0140-6736(91)91772-M.

Luther, J., R. Aitken, J. Milne, M. Matsuzaki, L. Reynolds, D. Redmer, and J. Wallace. 2007. Maternal and Fetal Growth, Body Composition, Endocrinology, and Metabolic Status in Undernourished Adolescent Sheep1. Biol. Reprod. 77:343–350. doi:10.1095/biolreprod.107.061440. Available from: https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod.107.061440

Manners, M. J., and J. A. Stevens. 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. Br. J. Nutr. 28:113–127.

McCoard, S. A., W. C. McNabb, S. W. Peterson, S. N. McCutcheon, and P. M. Harris. 2000. Muscle growth, cell number, type and morphometry in single and twin fetal lambs during mid to late gestation. Reprod. Fertil. Dev. 12:319–327.

Meyer, A. M., and J. S. Caton. 2016. Role of the small intestine in developmental programming : impact of maternal nutrition on the dam and offspring. Adv. Nutr. 7:169–178. doi:10.3945/an.115.010405.169.

Meyer, A. M., J. J. Reed, K. A. Vonnahme, S. A. Soto-Navarro, L. P. Reynolds, S. P. Ford, B. W. Hess, and J. S. Caton. 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. J. Anim. Sci. 88:2410–2424. doi:10.2527/jas.2009-2220.

Molle, J. D. C., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, R. L. Atkinson, L. R. Miller, S. P. Ford, W. J. Means, J. S. Caton, and B. W. Hess. 2004. Effects of maternal nutrient restriction during early to mid-gestation on cow and fetal visceral organ measurements. J. Anim. Sci. 82:129.

Munroe, D. G., A. K. Gupta, F. Kooshesh, T. B. Vyas, G. Rizkalla, H. Wang, L. Demchyshyn, Z.-J. Yang, R. K. Kamboj, and H. Chen. 1999. Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. Proc. Natl. Acad. Sci. 96:1569–1573.

Nathanielsz, P. W. 2006. Animal models that elucidate basic principles of the developmental origins of adult diseases. ILAR J. 47:73–82. doi:10.1093/ILAR.47.1.73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16391433

Neville, T. L., D. A. Redmer, P. P. Borowicz, J. J. Reed, M. A. Ward, M. L. Johnson, J. B. Taylor, S. A. Soto-Navarro, K. A. Vonnahme, L. P. Reynolds, and J. S. Caton. 2010. Maternal dietary restriction and selenium supply alters messenger ribonucleic acid expression of angiogenic factors in maternal intestine, mammary gland, and fetal jejunal tissues during late gestation in pregnant ewe lambs. J. Anim. Sci. 88:2692–2702. doi:10.2527/jas.2009-2706.

Nguyen, C. A., Y. Akiba, and J. D. Kaunitz. 2012. Recent Advances in Gut Nutrient Chemosensing. 28–34.

Niinikoski, H., B. Stoll, X. Guan, K. Kansagra, B. D. Lambert, J. Stephens, B. Hartmann, J. J. Holst, and D. G. Burrin. 2004. Onset of small intestinal atrophy is associated with reduced intestinal blood flow in TPN-fed neonatal piglets. J Nutr. 134:1467–1474. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15173413%5Cnhttp://jn.nutrition.org/content/134/6/1467.full.pdf

Odle, J., R. T. Zijlstra, and S. M. Donovan. 1996. Intestinal effects of milkborne growth factors in neonates of agricultural importance. J. Anim. Sci. 74:2509–2522.

Osgerby, J. C., D. C. Wathes, D. Howard, and T. S. Gadd. 2002. The effect of maternal undernutrition on ovine fetal growth. J. Endocrinol. 173:131–141. doi:10.1677/joe.0.1730131.

Petersen, Y. M., D. G. Burrin, and P. T. Sangild. 2001. GLP-2 has differential effects on small intestine growth and function in fetal and neonatal pigs. Am. J. Physiol. Integr. Comp. Physiol. 281:R1986–R1993.

Petersen, Y. M., J. Elnif, M. Schmidt, and P. T. Sangild. 2002. Glucagon-like peptide 2 enhances maltase-glucoamylase and sucrase-isomaltase gene expression and activity in parenterally fed premature neonatal piglets. Pediatr. Res. 52:498.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:e45.

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

Pluske, J. R., M. J. Thompson, C. S. Atwood, P. H. Bird, I. H. Williams, and P. E. Hartmann. 1996. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br. J. Nutr. 76:409–22. doi:10.1079/BJN19960046. Available from: http://www.journals.cambridge.org/abstract_S0007114596001493%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/8881713

Qiu, X. S., T. T. Huang, Z. Y. Shen, H. Y. Deng, and Z. Y. Ke. 2005. Effect of early nutrition on intestine development of intrauterine growth retardation in rats and its correlation to leptin. World J. Gastroenterol. 11:4419–4422.

Radtke, F., and H. Clevers. 2005. Self-renewal and cancer of the gut: two sides of a coin. Science. 307:1904–1909. doi:10.1126/science.1104815.

Ramsanahie, A., M. S. Duxbury, T. C. Grikscheit, A. Perez, D. B. Rhoads, J. Gardner-Thorpe, J. Ogilvie, S. W. Ashley, J. P. Vacanti, and E. E. Whang. 2003. Effect of GLP-2 on mucosal morphology and SGLT1 expression in tissue-engineered neointestine. Am. J. Physiol. - Gastrointest. Liver Physiol. 285:G1345–G1352. doi:10.1152/ajpgi.00374.2002. Available from: http://ajpgi.physiology.org/lookup/doi/10.1152/ajpgi.00374.2002

Ramsanahie, A. P., U. V Berger, M. J. Zinner, E. E. Whang, D. B. Rhoads, and S. W. Ashley. 2004. Effect of glucagon-like peptide-2 (GLP-2) on diurnal SGLT1 expression. Dig. Dis. Sci. 49:1731–1737.

Ran, T., H. Li, Y. Liu, S. Tang, X. Han, M. Wang, Z. He, J. Kang, Q. Yan, Z. Tan, and C. Zhou. 2016. Expression of genes related to sweet taste receptors and monosaccharides transporters along the gastrointestinal tracts at different development stages in goats. Livest. Sci. 188:111–119. doi:10.1016/j.livsci.2016.04.010. Available from: http://dx.doi.org/10.1016/j.livsci.2016.04.010

Redmer, D. A., J. M. Wallace, and L. P. Reynolds. 2004. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domest. Anim. Endocrinol. 27:199–217. doi:10.1016/j.domaniend.2004.06.006.

Reed, J. J., M. A. Ward, K. Vonnahme, T. L. Neville, S. L. Julius, P. Borowicz, J. B. Taylor, D. A. Redmer, A. Grazul-Bilska, L. Reynolds, and J. Caton. 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.

Rehfeld, J. F. 2004. A centenary of gastrointestinal endocrinology. Horm. Metab. Res. = Horm. und Stoffwechselforsch. = Horm. Metab. 36:735–741. doi:10.1055/s-2004-826154.

Reynolds, L. P., P. P. Borowicz, J. S. Caton, K. A. Vonnahme, J. S. Luther, C. J. Hammer, K. R. Maddock Carlin, A. T. Grazul-Bilska, and D. A. Redmer. 2010. Developmental programming: the concept, large animal models, and the key role of uteroplacental vascular development. J. Anim. Sci. 88. doi:10.2527/jas.2009-2359.

Robinson, J. J. 1977. The influence of maternal nutrition on ovine foetal growth. Proc. Nutr. Soc. 36:9–16.

Rodrigues, F. A. de P., P. H. Q. S. de Medeiros, M. de M. G. Prata, and A. Â. M. Lima. 2016. Fisiologia da Barreira Epitelial Intestinal. In: Blucher, editor. Sistema Digestório: Integração Básico-Clínica. São Paulo. p. 441–478.

Roffler, B., A. Fäh, S. N. Sauter, H. M. Hammon, P. Gallmann, G. Brem, and J. W. Blum. 2003. Intestinal Morphology, Epithelial Cell Proliferation, and Absorptive Capacity in Neonatal Calves Fed Milk-Born Insulin-Like Growth Factor-I or a Colostrum Extract1. J. Dairy Sci. 86:1797–1806. doi:https://doi.org/10.3168/jds.S0022-0302(03)73765-5. Available from:

http://www.sciencedirect.com/science/article/pii/S0022030203737655

Rosa, H. J. D., and M. J. Bryant. 2003. Seasonality of reproduction in sheep. Small Rumin. Res. 48:155–171. doi:https://doi.org/10.1016/S0921-4488(03)00038-5. Available from: http://www.sciencedirect.com/science/article/pii/S0921448803000385

De Santa Barbara, P., G. R. Van Den Brink, and D. J. Roberts. 2003. Development and differentiation of the intestinal epithelium. Cell. Mol. Life Sci. C. 60:1322–1332.

Sato, S., R. Hokari, C. Kurihara, H. Sato, K. Narimatsu, H. Hozumi, T. Ueda, M. Higashiyama, Y. Okada, C. Watanabe, S. Komoto, K. Tomita, A. Kawaguchi, S. Nagao, and S. Miura. 2013. Dietary lipids and sweeteners regulate glucagon-like peptide-2 secretion. AJP Gastrointest. Liver Physiol. 304:G708–G714. doi:10.1152/ajpgi.00282.2012. Available from: http://ajpgi.physiology.org/cgi/doi/10.1152/ajpgi.00282.2012

Sauter, S. N., B. Roffler, C. Philipona, C. Morel, V. Rome, P. Guilloteau, J. W. Blum, and H. M. Hammon. 2004. Intestinal Development in Neonatal Calves: Effects of Glucocorticoids and Dependence on Colostrum Feeding1. Neonatology. 85:94–104.

Scholljegerdes, E. J., K. A. Vonnahme, J. D. C. Molle, V. Nayigihugu, S. L. Lake, R. L. Atkinson, P. A. Ludden, L. R. Miller, S. P. Ford, and W. Hess. 2004. EFFECTS OF MATERNAL UNDERNUTRITION FROM EARLY TO MID-GESTATION ON VISCERAL ORGANS OF THE EWE AND FETUS.

Shirazi-Beechey, S. P. 1995. Molecular biology of intestinal glucose transport. Nutr. Res. Rev. 8:27–41. doi:10.1079/NRR19950005. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19094278

Shirazi-Beechey, S. P., B. A. Hirayama, Y. Wang, D. Scott, M. W. Smith, and E. M. Wright. 1991. Ontogenic development of lamb intestinal sodium-glucose co-transporter is regulated by diet. J. Physiol. 437:699–708. doi:10.1113/jphysiol.1991.sp018620.

Simmen, F. A., K. R. Cera, and D. C. Mahan. 1990. Stimulation by colostrum or mature milk of gastrointestinal tissue development in newborn pigs. J. Anim. Sci. 68:3596–3603.

Steinhoff-Wagner, J., S. Gors, P. Junghans, R. M. Bruckmaier, E. Kanitz, C. C. Metges, and H. M. Hammon. 2011. Intestinal Glucose Absorption but Not Endogenous Glucose Production Differs between Colostrum- and Formula-Fed Neonatal Calves. J. Nutr. 141:48–55. doi:10.3945/jn.110.128652. Available from: http://jn.nutrition.org/cgi/doi/10.3945/jn.110.128652

Stephens, J., B. Stoll, J. Cottrell, X. Chang, M. Helmrath, and D. G. Burrin. 2006. Glucagon-like peptide-2 acutely increases proximal small intestinal blood flow in TPN-fed neonatal piglets. Am. J. Physiol. Integr. Comp. Physiol.

Swanson, T. J., C. J. Hammer, J. S. Luther, D. B. Carlson, J. B. Taylor, D. A. Redmer, T. L. Neville, J. J. Reed, L. P. Reynolds, J. S. Caton, and K. A. Vonnahme. 2008. Effects of gestational plane of nutrition and selenium supplementation on mammary development and

colostrum quality in pregnant ewe lambs. J. Anim. Sci. 86:2415–2423. doi:10.2527/jas.2008-0996.

Symonds, M. E., S. P. Sebert, and H. Budge. 2010. Nutritional regulation of fetal growth and implications for productive life in ruminants. Animal. 4:1075–1083. doi:10.1017/S1751731110000479. Available from: http://www.journals.cambridge.org/abstract_S1751731110000479

Symonds, M. E., T. Stephenson, D. S. Gardner, and H. Budge. 2007. Long-term effects of nutritional programming of the embryo and fetus: Mechanisms and critical windows. Reprod. Fertil. Dev. 19:53–63. doi:10.1071/RD06130.

Taylor-Edwards, C. C., D. G. Burrin, J. C. Matthews, K. R. Mcleod, and J. J. Holst. 2010. Expression of mRNA for proglucagon and glucagon-like peptide-2 (GLP-2) receptor in the ruminant gastrointestinal tract and the influence of energy intake. DAE. 39:181–193. doi:10.1016/j.domaniend.2010.05.002. Available from: http://dx.doi.org/10.1016/j.domaniend.2010.05.002

Tian, S., J. Wang, H. Yu, J. Wang, and W. Zhu. 2018. Effects of galacto-oligosaccharides on growth and gut function of newborn suckling piglets. 1–11.

Tivey, D., J. Le Dividich, P. Herpin, D. Brown, and M. Dauncey. 1994. Differential effects of lipid and carbohydrate on enterocyte lactase activity in newborn piglets. Exp. Physiol. 79:189–201. doi:10.1113/expphysiol.1994.sp003752.

Trahair, J. F., T. M. DeBarro, J. S. Robinson, and J. A. Owens. 1997. Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. J. Nutr. 127:637–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9109616

Trahair, J. F., and P. T. Sangild. 2002. Chapter 1 Studying the development of the small intestine: philosophical and anatomical perspectives. Elsevier Ltd. Available from: http://dx.doi.org/10.1016/S1877-1823(09)70117-1

Tsai, C. H., M. Hill, S. L. Asa, P. L. Brubaker, and D. J. Drucker. 1997. Intestinal growth-promoting properties of glucagon-like peptide-2 in mice. Am. J. Physiol. 273:E77-84. doi:10.1152/ajpendo.1997.273.1.E77.

Ulluwishewa, D., R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, and N. C. Roy. 2011. Regulation of tight junction permeability by intestinal bacteria and dietary components. J. Nutr. 141:769–776.

Umar, S. 2010. Intestinal stem cells. Curr. Gastroenterol. Rep. 12:340–348. doi:10.1007/s11894-010-0130-3.

Vonnahme, K. A., M. J. Zhu, P. P. Borowicz, T. W. Geary, B. W. Hess, L. P. Reynolds, J. S. Caton, W. J. Means, and S. P. Ford. 2007. Effect of early gestational undernutrition on angiogenic factor expression and vascularity in the bovine placentome. J. Anim. Sci. 85:2464–2472. doi:10.2527/jas.2006-805.

Wang, J., L. Chen, D. Li, Y. Yin, X. Wang, P. Li, L. J. Dangott, W. Hu, and G. Wu. 2008. Intrauterine growth restriction affects the proteomes of the small intestine, liver, and skeletal muscle in newborn pigs. J. Nutr. 138:60–6. doi:138/1/60 [pii]. Available from: http://jn.nutrition.org/content/138/1/60.full

Wang, T., J. H. Yong, F. Shi, J. X. Ruo, and R. J. Hutz. 2005. Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. Biol. Neonate. 88:66–72. doi:10.1159/000084645.

Watson, R. R., D. N. McMurray, and D. C. Bauer. 1979. The effects of malnutrition on secretory and cellular immune processes. C R C Crit. Rev. Food Sci. Nutr. 12:113–159. doi:10.1080/10408397909527275. Available from: http://dx.doi.org/10.1080/10408397909527275

Wayne, N. L., B. Malpaux, and F. J. Karsch. 1989. Social cues can play a role in timing onset of the breeding season of the ewe. J. Reprod. Fertil. 87:707–713. doi:10.1530/jrf.0.0870707.

Weaver, L. T., S. Austin, and T. J. Cole. 1991. Small intestinal length: a factor essential for gut adaptation. Gut. 32:1321–1323. doi:10.1136/gut.32.11.1321.

Wright, N. A. 2000. Epithelial stem cell repertoire in the gut: clues to the origin of cell lineages, proliferative units and cancer. Int. J. Exp. Pathol. 81:117–143.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316–2337. doi:10.2527/jas.2006-156.

Xu, R. J., P. T. Sangild, Y. Q. Zhang, and S. H. Zhang. 2002. Chapter 5 Bioactive compounds in porcine colostrum and milk and their effects on intestinal development in neonatal pigs11This work has been supported by the Research Grants Council of the Hong Kong Special Administrative Region, China (HKU 7234/98M). In: R. Zabielski, P. C. Gregory, B. Weström, and E. B. T.-B. of G. A. Salek, editors. Biology of the Intestine in Growing Animals. Vol. 1. Elsevier. p. 169–192. Available from: http://www.sciencedirect.com/science/article/pii/S1877182309701213

Yu, C., G. Jia, Y. Jiang, Q. Deng, Z. Chen, and Z. Xu. 2014. Effect of Glucagon-like Peptide 2 on Tight Junction in Jejunal Epithelium of Weaned Pigs though MAPK Signaling Pathway. 27:733–742.

Yusta, B., L. Huang, D. Munroe, G. Wolff, R. Fantaske, S. Sharma, L. Demchyshyn, S. L. Asa, and D. J. Drucker. 2000. Enteroendocrine localization of GLP-2 receptor expression in humans and rodents. Gastroenterology. 119:744–755.

Zhang, H., L. Dong, M. Wang, L. Yu, and H. Wang. 2018. Effects of dietary L-arginine and N-carbamylglutamate supplementation on intestinal integrity , immune function and oxidative status in intrauterine growth retarded suckling lambs. doi:10.1021/acs.jafc.8b00726.

Zhang, Y., X. Zhang, X. Liu, Y. Li, and J. Ding. 2013. Reference Gene Screening for Analyzing Gene Expression Across Goat Tissue. 26:1665–1671.

Zhong, Y., T. Saitoh, T. Minase, N. Sawada, K. Enomoto, and M. Mori. 1993. Monoclonal antibody 7H6 reacts with a novel tight junction-associated protein distinct from ZO-1, cingulin and ZO-2. J. Cell Biol. 120:477–483.