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Developmental Programming in Dairy Heifers: effects of maternal energy restriction or overfeeding in dairy heifers during early gestation on the development of the gastrointestinal tract in their offspring

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Developmental Programming in Dairy Heifers: effects of maternal energy restriction or overfeeding in dairy heifers during early gestation on the development of the gastrointestinal tract in their offspring

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DEDICATION

This thesis is dedicated to my family and to my grandad of blessed memory.

DECLARATION

I declare that this thesis and the results contained therein are my original thoughts and have never been submitted to another institution for consideration for a PhD or a degree of a similar nature.

David O. Edache

RESEARCH BACKGROUND

The environment encountered during fetal life influences development and post-natal risk of impaired visceral gastrointestinal or immune organ development in heifers with disadvantageous effects on animal welfare and economy. As far back the late 80s, the Barker hypothesis was established. The main findings from David J. Barker's study were that human offspring born to pregnant mothers exposed to periods of starvation during the second world war (Dutch winter famine) had low birth weight, usually associated with chronic, degenerative conditions of adult health, including heart disease and type 2 diabetes, may be triggered by circumstances decades earlier, *in utero* nutrition. Furthermore, the pioneering work at Barker's laboratory also showed that babies born small for their gestational age were more likely to die from cardiac diseases in adulthood. Using animal models, these conditions have shown a potential to be transferred through multigeneration. This set the stage for studies on developmental programming or sometimes referred to as fetal programming and allow the cattle industry to rethink its nutritional management of heifers during pregnancy. In fetal life, the placental plays a key role in the transfer of nutrients from dam to fetus. However, when postnatal feeding commences, a well-developed gastrointestinal tract is required. For this reason, adequate development of the gastrointestinal tract from the uterine environment is the goal of several fetal programming studies. Besides the established evidence that alterations in maternal nutrition or perturbations from other environmental stressors can reprogram fetal organs in adulthood, the timing and length of the insult is critical. So far, evidence from sheep and dairy cows show that as early as d 30-45 of pregnancy, most organs including the gastrointestinal tract are established, even though fetal organ systems continue to grow, develop and differentiate throughout gestation. Less is understood regarding the effects of early gestation maternal energy restriction in dairy heifers on gastrointestinal tract development in their offspring. Majority of the studies have focused on late gestation heat stress on immune and gastrointestinal tract organ development in ruminants. Furthermore, data on developmental programming from these studies have mostly stopped either at the fetal life or between birth and weaning. In addition, the breed, sex, age or timing of the intrauterine insult have been inconsistent in literature. Herein, this study tested the hypothesis that early gestation maternal energy restriction in dairy heifers will produce offspring with underdeveloped gastrointestinal tract later in life. To test this hypothesis, a study was conducted to investigate the effects of feeding dairy heifers a limited amount of their maintenance energy requirement from shortly before conception to early gestation on the development of the gastrointestinal tract in their offspring. The specific objectives evaluated in this study were dealt with in Chapters contained in this thesis.

GENERAL SUMMARY

Holstein Friesian heifers (n=28; initial BW±SD; 371.57±42.14, age±SD; 15.7±1.08 months) homogenous for body weight and age were randomly assigned into three experimental groups and, from 11 days before artificial insemination were individually fed as following: (i) 0.6 M of their maintenance energy requirements (M) until day 80 of gestation (nutrient restricted NR80, n=11), (ii) 0.6 M until day 120 of gestation (NR120, n=11), and (iii) ad libitum (1.8 M) until day 120 of gestation (Control, n=6). Heifers were group fed once the differential feeding period ended, with ad libitum access to feed until calving. Twenty-two single female calves were retained after calving. All calves received the same management and diet until slaughter (BW±SD; 117±13). Four days until slaughter, calves were housed individually and received the same management and diet. Calves were euthanised at d 135±3 to obtain organs weight. Data analysis on maternal and calf variables were performed in R. Overall results on pregnant heifers presented in Chapter 3 showed that maternal growth performance during pregnancy depends on the nutritional status of the heifer from preconception to early gestation. However, nutrient restricted heifers in early gestation showed capacity for catch up growth and compensated for nutrient losses evidenced from improved dry matter intake, average daily gain, rumination time and apparent total tract digestibility as pregnancy progressed. Furthermore, regardless of nutritional group, apparent total tract digestibility reduced in the last days to calving having piqued at d 200 of gestation for all nutrients evaluated. Gestation length was similar across nutritional groups with similar BCS at calving. In Chapter 4, data on the phenotypic, behavioural and digestive performances of pre-weaned calves were presented. Results showed that calves born to overfed mothers during early gestation were heavier at birth compared to their peers (P=0.017). In addition, they had larger thoracic circumference and stature than calves born to nutrient restricted mothers during early gestation (P=0.049). However, as calves aged, these differences disappeared. Despite the low birth weight in NR80 calves, they had the highest starter intake and rumination time at preweaning (P<0.05). Yet, apparent nutrient digestibility remained similar among all calves. Similarly, DMI, apparent total tract digestibility and body weight gains from weaning until slaughter remained similar among all calves. However, time spent feeding continued to be higher for NR80 calves than their peers (P<0.05). In Chapter 5, results on the development of the gastrointestinal tract and digestive physiology of post weaned dairy calves were discussed. GIT organ weights were similar among all calves except for the weight of the duodenum that tended to be higher in calves from control fed mothers than their counterparts. Sieve analysis on particle size distribution along the GIT showed normal digestive physiology as higher dry matter of digesta was observed in the foregut than the lower gut. Similarly, higher mean particle size of digesta was recorded in digesta from the lower gut on sieve sizes less than 1.18mm. Despite the fact that calves showed the capacity to undergo catch up growth both in body and organ outcomes, the development of the duodenum in offspring from NR80 fed heifers may be impaired. Continued research is necessary to investigate if this impairment would persist later in life with a potential detrimental effect on their productive performance as replacement heifers. The implications of these results are that *in utero* maternal nutrient restriction in early gestation may produce calves with underdeveloped duodenum and potentially limit the capacity of the duodenum to secrete requisite enzymes necessary to hydrolyze glycosidic linkage of saccharides required to enhance nutrient digestion and absorption.

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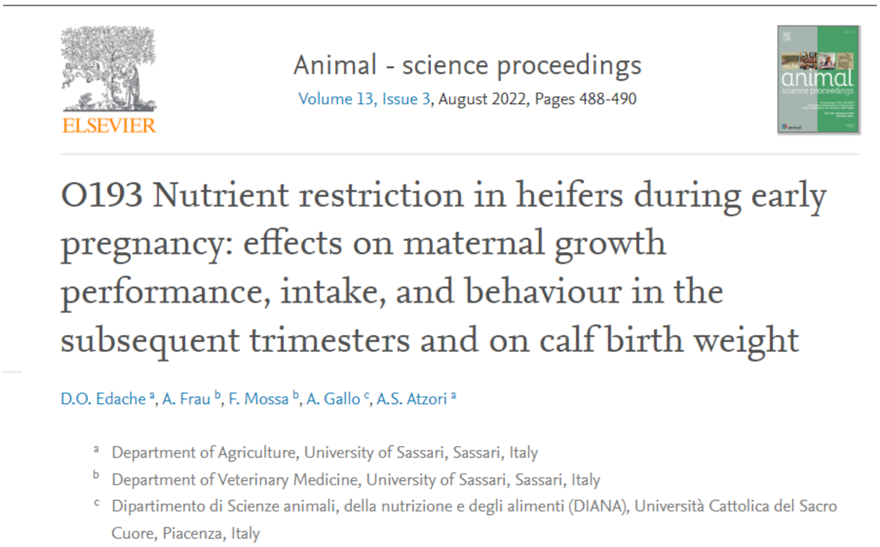

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O193 Nutrient restriction in heifers during early pregnancy: effects on maternal growth performance, intake, and behaviour in the subsequent trimesters and on calf birth weight

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82. Cattle animal model of fetal programming to study effects of early gestation nutrient restriction on growth performance, intake, and behavior of pre-weaned calves

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LIST OF ABBREVIATIONS

ADIP	Acid detergent insoluble protein
ADF	Acid detergent fibre
CP	Crude protein
DMI	Dry matter intake
DM	Dry matter
DoHAD	Developmental origin of Health and Diseases
DG	Day of gestation
dMEAN	Discrete mean
EE	Ether extract
FP	Feeding program
GIT	Gastrointestinal tract
IUGR	Intrauterine growth restriction
NR	Nutrient restriction
NDIP	Neutral detergent insoluble protein
NR120	Nutrient restricted from 11 days preconception to d 120 of pregnancy
NR80	Nutrient restricted from 11 days preconception to 80 days of pregnancy
NFC	Non fibrous carbohydrates
PeNDF	Physical effective neutral detergent fibre
SI	Selectivity index
SOLP	Soluble protein

CHAPTER 1

Developmental Programming of the Fetal Gastrointestinal Tract in Ruminants: A review

ABSTRACT

Alterations in maternal nutrition during gestation appear to regulate fetal gastrointestinal tract (GIT) development and shape offspring postnatal performance by altering organ and tissue development. The activities of the GIT in ruminants can be programmed throughout fetal life, just like the placenta and other fetal organs. In ruminants, fetal GIT development begins early in gestation and varies throughout pregnancy, with accelerated fetal GIT growth observed in the third trimester. However, due to a lack of information in the literature, the short- and long-term biological consequences of altered fetal GIT development are not yet fully uncovered. Nonetheless, the GIT must have a sufficient surface area and capacity for digestion and nutrition absorption when enteral or postnatal feeding begins, as it is one important functional and crucial metric for healthy development *in utero*. In this review, we evaluated the scientific literature on the development of the fetal gastrointestinal tract in ruminants and the impact of maternal over- and undernutrition on the development of the GIT in their offspring. We contend that maternal nutrient restriction and overfeeding, regardless of gestational stage, can alter fetal GIT development and set the stage for future worries about the GIT's functional capabilities. In addition, we applied a system thinking approach to conceptualizing maternal nutrient restrictions during pregnancy on overall herd productivity. In order to create a program for fetal GIT development in ruminants that is effective, nutritional techniques that can be used during gestation to enhance the intrauterine environment must be identified. This review outlined some critical knowledge gaps where additional research could advance our understanding of how the timing, duration, and severity of nutritional programming during pregnancy might affect the future GIT function, capacity, and performance of heifers born to mothers exposed to nutritional stress during pregnancy. This limitation was based on the paucity of studies that have attempted to investigate the long-term effects of maternal nutritional programming on ruminant growth and development of fetal GIT.

Highlights

- The intrauterine environment is crucial for the formation of the gastrointestinal tract, which depends on maternal supplies for adequate growth and development.
- In Ruminants, the activities of the gastrointestinal tract can be programmed from prenatal life.
- Maternal nutrition during pregnancy may influence fetal GIT development as early as the first trimester of pregnancy.
- The short- and long-term biological consequences of altered fetal GIT development are not yet well understood.
- Fetal GIT development is influenced by the stage of pregnancy and the timing of the insults.

Keywords: GIT programming, Fetal, Maternal nutrition, Gestation, Ruminants

1. INTRODUCTION

Interest has recently grown in the intrauterine life of mammals because evidence indicates that several phenotypes of postnatal life in livestock can be influenced by conditions occurring during fetal development; these include growth performance (Martin et al., 2007; Gardner et al., 2007; Kenyon and Blair, 2014; Fraga et al., 2018) and nutrient partitioning (Caton et al., 2019; Lunesu et al., 2020), as well as health and fertility (Rae et al., 2002; Mossa et al., 2018; Hoffman et al., 2018). This concept has been referred to as fetal programming (Barker, 1990; 2004) or developmental programming (Reynolds et al., 2010) and was initially based on evidence from an epidemiological study conducted by Dr. David J. Barker in humans, which investigated how the uterine environment of women that were pregnant during the Dutch winter famine (World War II) affected their progeny (Ravelli et al., 2008).

The main findings from David J. Barker's study were that low birth weight, usually associated with chronic, degenerative conditions of adult health, including heart disease and type 2 diabetes, may be triggered by circumstances decades earlier, *in utero* nutrition. Furthermore, the pioneering work at Barker's laboratory also showed that babies born small for their gestational age were more likely to die from cardiac diseases in adulthood (Barker, 1990). This inspired the phrase 'The Barker hypothesis' and set the stage for continued research in the developmental origin of health and diseases (DoHAD).

Generally, the term 'fetal programming' is broadly used to indicate the impact of factors (i.e., nutritional imbalances, endocrine disruptors, environmental conditions) that may trigger a response in the developing conceptus and predetermine a long-term effect in postnatal life

(Langley-Evans and McMullen, 2010). However, these effects may be detectable at birth as the individual grows and develops (Godfrey and Barker, 2000).

Based on this premise, the term 'developmental programming' has been argued to be more accurate than 'fetal programming' since growing evidence indicates that environmental stimuli in early pregnancy have shown long lifetime repercussions (Meyer and Caton, 2016; Vautier and Cadaret, 2022). Indeed, environmental and nutritional stimuli occurring before birth can permanently program physiological systems due to the high plasticity of the developing fetus (Paliy et al., 2014).

Aside from the human origins of the concept of fetal or developmental programming, insufficiencies during pregnancy have been extensively studied in a variety of other species, including laboratory animals (mice model; Aguila et al., 2021) and domesticated livestock such as ruminants (Caton et al., 2009; Mossa et al., 2013; Nascimento et al., 2022). Given that rodents have long been used as the first step in biomedical research before introducing human research, there are recommendations from the American Institute of Nutrition (AIN) to model fetal programming and induce cardiovascular and chronic diseases in adulthood from intrauterine perturbed mothers or fathers in rodents (Reeves et al., 1993).

In ruminants, the gastrointestinal tract (GIT) consists of a series of tubular organs and associated glands that break down ingested food into smaller units, regulate metabolism and absorption of nutrients, and protect the host from the constantly fluctuating combination of toxins, chemicals, and microorganisms in the lumen (Gaebel et al., 2001). This is achieved by the rapid development of the GIT in early life (Trahair et al., 1997).

Infants with intrauterine growth retardation have a substantially higher chance of getting sick (Gruenwald 1963). Mucosal immunity suffers a reduction in immune function (Watson and McMurray 1979) in new-borns with low birth weight and inadequate nutrition. In the U.S., mortality and morbidity rates of 10% and more than 46%, respectively, were reported in preweaning dairy calves, with pathologies of GIT being prevalent (USDA, 2007). In another survey on commercial dairy farms in Canada (Ontario) and Minnesota (USA), the first illness in a calf's life was found to be due to gastrointestinal infections, with ~23% of these calves requiring antibiotic diarrhea treatment (Windeyer et al., 2014).

Additionally, in ruminants, the activities of the GIT can also be programmed during prenatal life, and this finding is significant because the GIT serves as a primary site for nutrient uptake, secretion of hormones, and excretion of waste (Connor et al., 2010). According to Baldwin et al. (2004), the ruminant GIT can adapt to changes in both physiology and metabolism. This adaptive mechanism is significant because the portal-drained viscera account for about 20% of the animal's total oxygen use (Meale et al., 2017). Additionally, the calf GIT, known to be somewhat plastic and capable of recognizing nutrient supply and responding to bioactive compounds, has shown this adaptive process in action (Meale et al., 2017).

Much of the GIT changes occur between birth and weaning, which have been associated with reduced growth, GIT impairments, and overall poor animal efficiency (Wang et al., 2008; Khan et al., 2011). Despite this knowledge, the biological consequences of altered fetal GIT development in the short and long term are still poorly understood.

The GIT of young ruminants undergoes significant development *in utero*, such that much of their architectural and structural functions are established before birth (Warner et al. 1956).

The characteristics of the structural capacities of the GIT vary (i.e., a marked difference between the lower intestine and the rumen, reticulum, and omasum), and this can be explained by either (a): The presence of a simple columnar epithelium (CE) rather than a multi-layered squamous epithelium (SSE) in the lower intestine and (b): the cells of the lower intestine include the absorptive epithelium, enteroendocrine cells, immune cells (e.g., dendritic cells, Paneth cells, and lymphocytes), and mucus-secreting cells (goblet cells) (Peterson and Artis, 2014).

The functions of these specialized cells include 1). Secretion of protective substances into the lumen, 2). Enzyme secretion into the lumen, 3). Facilitation of nutrient uptake and secretion of hormones [e.g., glucagon-like peptide (GLP)-1 and GLP-2] (Lu et al., 2021). Regulatory peptides, plasma, and luminal concentrations in the gut significantly influence GIT development in ruminants (Guilloteau et al., 2009).

Most of the data on fetal GIT programming in ruminants, albeit limited, comes from perturbations in maternal nutrition and has been shown to cause impairment of total fetal GIT weight (Meyer et al., 2010), reduced enzymatic activities (Trotta et al., 2020) or altered digestive capacity in offspring (Noya et al., 2019). It is crucial to consider the combination of materno-fetal nutritional stress, timing, and length at which pregnant ruminant animals are exposed to nutritional stress to create a model of developmental programming for the ruminant livestock industry.

Therefore, this review evaluates the existing scientific literature on fetal GIT development and the effects of maternal over- and undernutrition during pregnancy on offspring GIT development in ruminants.

1.2. Intrauterine developments of the gastrointestinal tract

Figure 1 demonstrates the progression of fetal organ development throughout pregnancy. Besides the development of the placenta during the early stages of fetal development, other critical events occur during pregnancy (Funston et al., 2010). These processes (adipogenesis, fibrogenesis, and myoblast proliferation) occur gradually and depend on the nutrients the conceptus receives from the dam, which is particularly pronounced in muscle fiber development (Du et al., 2010; 2017; Costa et al., 2021). Several studies have shown that intrauterine growth restriction (IUGR) of the fetus results in reduced fetal growth, lowers birth weight, and limits the lifetime performance of the offspring (Godfrey and Barker, 2000; Wu et al., 2006; Caton and Hess, 2010). Therefore, continued research should focus on developing management techniques to prevent maternofetal stress during gestation as selection and breeding criteria shift toward informed decision-making based on the developmental origin of health and disease (DoHAD) history.

The critical events for normal embryo development begin in the earliest stages of gestation. These include fetal organogenesis, vascularity, differentiation, and placenta development (Funston et al., 2010). In beef cows, both fetal organs and the placenta develop concurrently, and as early as day 25 of gestation, limbs are formed (Summers and Funston, 2013). Interestingly, Hubbert et al. (1972) found that after limb formation, the pancreas, liver, adrenal glands, lungs, thyroid, spleen, brain, thymus, and kidneys develop in chronological order, and these organs; Kidney (Gilbert et al., 2007), heart (Han et al., 2004; Wang et al., 2011), pancreas (Limesand et al., 2006), lungs (Clifton et al., 2016), placenta (Redmer et al., 2004; Reynolds et al., 2010; Micke et al., 2010) and small intestine (Greenwood and Bell, 2003; Meyer and Caton, 2016) have been studied in livestock fetal programming models.

The normal, expected growth of the fetal ruminant GIT is achieved when fetal body growth is unaffected from conception to postnatal life (Xu et al., 1994; Vonnahme et al., 2003; Soto-Navarro et al., 2004). However, long-term reduction in oxygen and nutrient delivery to the growing fetus can restrict its growth (Harding and Johnson, 1995) and consequently delay the development GIT, evidenced in sheep (Avila et al., 1989).

Several time windows are critical for the development of the GIT during fetal, perinatal, and neonatal life (Nathanielsz, 2006), as organogenesis usually occurs in early to mid-pregnancy and is followed by rapid fetal growth in the last third of gestation before being prepared for the transition from the uterus to the external environment during the perinatal period (Fowden et al., 2006).

The first third of gestation in both pigs and sheep is for the anatomical formation of the fetal GIT, while the second and last third of pregnancy represents the enzymatic and functional developmental phase of the fetal GIT, especially for the full development of small intestinal mobility, which is essential for the secretion and absorption of substances by the small intestine of the newborn (Couture, 2008). Because organogenesis occurs in early to mid-pregnancy (Meyer et al., 2010), the functional alteration of fetal gastrointestinal organs during early pregnancy feed restriction may be fundamentally more severe (Trotta and Swanson, 2021).

As reported by several authors, the onset of fetal GIT development varies in ruminant species and other related species characterised by a long gestation period or similar digestive microbiome (Table 1). The fetal GIT development follows a unique pattern of increased growth in the third half of gestation, which confirms claims that more than 75% of fetal organ development happens in this period (Stalker et al., 2006; Guilloteau et al., 2009; Duarte et al., 2013).

For instance, a study in cattle revealed that from day 175 to day 280 of pregnancy, the fetal's small intestine grew twice as fast as the rest of the body (Dobson et al., 1984), while another study in sheep found that the viscera grew cubically with increasing gestation length (Osgerby et al., 2002), suggesting that more significant development takes place in the later stages of pregnancy.

In general, GIT development is accelerated primarily by conversion from parenteral nutrition to total enteral nutrition (Sangild et al., 2002), although evidence of enteral nutrition before birth has also been documented (Mulvihill et al., 1985). In the first half of gestation, the fetus GIT receives nutrients from maternal blood via the placenta, whereas in mid to late pregnancy, it receives enteral nutrients by ingesting amniotic fluid (usually ~20% of body weight per day) (Mulvihill et al., 1985). In fetal sheep, fluid intake in late gestation ranges from 100 to 1000 ml per day (Harding et al., 1984; Tomoda et al., 1985), whereas the human fetus can swallow up to 750 ml per day (Ross and Nijland, 1998).

Amniotic fluid, although described as less nutritious, contains free amino acids, proteins, and some growth factors that provide 10-20% of fetal energy requirements (Mulvihill et al., 1985) and 10-15% of fetal nitrogen requirements (Pitkins and Reynolds, 1975), and these fluids are swallowed along with pulmonary secretions (Hardy et al., 1984). This exclusive form of digestion depends on the development of endosomal capacity and lysosomal activity (Trahair et al., 1997), and this indicates that fetal ingestion of fluids can initiate the development of the gastrointestinal tracts in ruminants.

In addition, the mechanism of fetal swallowing, which is enhanced by the transport of key nutrients into the amniotic fluid (namely glutamine, arginine, proteins, hormones, vitamins, growth factors, and minerals) during late gestation, is vital in preparing the GIT for postnatal nutrition (Underwood and Sherman, 2006).

Ruminants and humans with long gestation experience the most rapid phase of GIT development in late gestation (Weaver et al. 1991). However, defective development of GIT, particularly mucosal tissues of growth-restricted fetal sheep and pigs, has also been reported to occur in late gestation (Avila et al., 1989; Xu et al., 1994). As mentioned earlier, normal development of GIT continues after birth, which is possible through the epigenetic mechanism of gene regulation in the gut, as shown by the ontogeny of gut enzymes and hormones in rodents (Gartner et al., 2002).

Most studies on nutrient transfer during gestation from the dam to fetus have focused on the uteroplacental unit (Reynolds et al., 2010; Vonnahme, 2012), and very little information is available on the development and capacity of the fetal GIT, especially in ruminants (Trahair et al., 1997; Duarte et al., 2013). It can be speculated that potential causes of the placenta's extensive research include (a) the organ's remarkable ability to deliver nutrients and respiratory gases through a transplacental exchange from the mother to the fetus (Wu et al., 2004) or (b) the association between the weight of the calf at birth and the total placental weight at calving (Echternkamp, 1993).

It is not fully understood if certain intrauterine stress during pregnancy can affect the development and functions of the foregut, especially the abomasum. Ruminants' digestive systems are said to be nonfunctional from birth until the establishment of the rumen, allowing liquid nutrition (mostly colostrum, whole milk, and milk replacer) to enter the abomasum directly. This is made possible by obstruction of the esophageal groove (Burgstaller et al., 2017; Diao et al., 2019).

Depending on the type of ruminant, rumen histomorphogenesis begins as early as 30 to 35 days into the fetal life, and stratification of the rumen epithelium occurs after day 50 of gestation, corresponding with the development of ruminal pillars and papillae (García et al., 2012). As a weaning indicator, rumen histomorphogenesis is necessary for ruminants during their postweaning phase.

1.3. Fetal Development of the four gastric compartments in ruminants

Fetal development of the abomasum and small intestine is necessary for efficient nutrient absorption and use before rumen development. The fetal small and large intestines and the four stomach compartments were clearly visible on the day of calving (Duarte et al., 2013). Additionally, the abomasum and omasum developed before the reticulo-rumen, and the small intestine preceded the colon. This demonstrates the significance of the small intestine and abomasum for nutritional absorption and the fact that they have a higher developmental priority than the other GIT organs (Duarte et al., 2013). There are variations in the growth and development of the specialized distinct forestomach compartments (from conception to calving) in fetal cattle (Figure 1.2).

Although the rumen appeared to develop first during the first 150 days of pregnancy (Figure 1.2), the pace of development of the forestomach was identical. The abomasum, however, ranked first as gestation progressed and at full term since it is the GIT organ the neonate needs the most during the first few weeks of life (Becker et al., 1951; Fubini and Ducharme, 2004). In fact, the findings from Meyer et al. (2010), using the bovine model, showed that fetal cattle had a greater mass of these primitive gastric organs per unit of body weight at d 125 of gestation. Furthermore, the entire GIT, reticulo-rumen, abomasum, and liver grew faster at the beginning than at the end of pregnancy (Meyer et al., 2010). To some degree, these studies show that the postnatal development of the GIT is a continuation of its differentiation and structure initiated in prenatal life. Whether some GIT organs in ruminants attain their final anatomic and morphometric architecture before parturition is less understood. Evidence from dairy calves born to mothers exposed to late gestation heat stress showed no difference in major gastrointestinal tract organs at weaning (Dado-Senn et al., 2021).

Although knowledge of prenatal development of the four gastric compartments in cattle and cows is limited, there is far less information in the literature regarding prenatal forestomach development in sheep and goats. Gao et al. (2008) attempted to quantify the weights of each gastric compartment in sheep in kilograms and reported that the abomasum was the heaviest (20.85 g) in day-old lambs compared to the other compartments (5.86 g and 2.44 g for reticulo-rumen and omasum, respectively).

The data on sheep and cows reported in this review demonstrate the importance of proper development of the abomasum for immediate postnatal survival of ruminants. In the abomasum, the digestive enzymes required for the breakdown of ingested dietary components (during the first months of life) are predominantly secreted in this gastric compartment (Masot et al., 2007), and this could further explain the developmental priority of this organ.

1.3.1. *Small Intestine*

The small intestine has been the focus of many studies of fetal GIT programming because of its role in nutrient absorption, utilization, and immunocompetence (Van der Meer et al. 2016). The fetus' small intestine is relatively short compared to its maternal counterpart, and fetal intestinal development and cellular differentiation begin during the first third of gestation (Trotta and Swanson, 2021). During this period, linear growth of the small intestine occurs, reaching its final anatomical position in the abdomen after additional coelomic expansion and rotation until 20 weeks of gestation (Nagata et al., 2019). Thereafter, accelerated longitudinal growth occurs until parturition.

After birth, growth of the small intestine continues slower until adulthood. In ruminants, the small intestine consumes approximately 20% of maintenance energy (Caton et al., 2000) and is, therefore, one of the first organs affected by limited feeding or other intrauterine insults. Small intestinal growth, development, and vascularization are essential processes that ensure immunological protection, nutrient metabolism, neonatal survival, and postnatal growth (Meyer and Caton, 2016). These processes have been thoroughly reviewed by Caton et al. (2019).

Due to the highly plastic nature of the small intestine, it can change with nutrient uptake and physiological state even in adulthood, and the maternal small intestine adapts to pregnancy and progressive gestation (Meyer and Caton, 2016) or the environment to which the animal is exposed (Trahair and Sangild, 2002). As the fetal intestine prepares for birth and is dependent on enteral nutrition, the development of its digestive and absorptive functions is stimulated by many factors, particularly amniotic fluid swallowing, and fetal cortisol production (Sangild et al., 2000).

Concerning intestinal immunity, it has been suggested that fetuses with IUGR are born with an immature small intestine (Trahair et al., 1997; Greenwood and Cafe, 2007), which may account for some of their reduced immune capacity (Cronjé, 2003), viability (Wu et al., 2006), and developmental potential (Caton et al., 2009; Greenwood and Cafe, 2007) during early neonatal survival. In contrast, a study by Meyer et al. (2010) showed that the small intestine and fetal weight were not affected by maternal nutrition, although the intestine of fetuses from restricted mothers appeared to compensate for this insult by increased proliferation and vascularization.

Several questions can be derived from this finding, such as whether the fetus' small intestine can physiologically adapt to changes during pregnancy or whether this adaptation is apparent only in the external environment when similar impairments are present.

1.4. Nutritional programming of the fetal gastrointestinal tract

The influence of nutrition on the development of ruminants' GIT during the early growth phase is important, primarily because the GIT oversees carrying nutrients to the body's cells in the first physiological step and is essential for controlling how new-born mammals develop (Guilloteau et al., 2009). In addition, the ruminant GIT accounts for a larger proportion of body weight compared to monogastric animals (Sun et al., 2013).

As mentioned earlier, in many nutritional studies, the bulk of the data has been gathered about the intrauterine environment (Fleming et al., 2004), placental adaptation (Myatt, 2006), and fetal/or cow nutrition (Jennings et al., 2015). Nutrition in early life can program fetal GIT and IUGR caused by disturbances in nutrition, such as energy restriction, delays GIT development and consequently impairs digestive functions evidenced in goats (McLeod and Baldwin, 2000; Shen et al., 2004).

Ruminants' failure to efficiently utilize every stomach compartment has several adverse effects on animal performance as well as returns on investments. For instance, in order to be used, all feed elements must be transformed into microbial nutrients by rumen microorganisms (Liu et al., 2021). Therefore, a well-developed rumen and the entire GIT are essential for nutrient metabolism, and animal survival and productivity.

The timing, duration, and extent of the imbalanced diet are important factors that may contribute to how the dam utilizes nutrients and further explain the severity of IUGR. Previously, Funston et al. (2010) reported that in ruminants, inadequate nutrition during early gestation might be less important because fetal nutrient requirements for growth and development are limited during the first half of pregnancy. These assumptions are not cohesive as there is emerging evidence that perturbations of maternal nutrition on fetal gut development in cattle may begin early in gestation (Duarte et al., 2013; Chu et al., 2017). As evidenced in sheep, a programming response on fetal α-amylase activity was observed when ewes were restricted to 60% of maintenance requirements during early (day 30 -85) pregnancy (Keomanivong et al., 2017a).

The above studies suggest that a change in nutrient requirement during the first half of gestation could significantly affect offspring performance and nutrient utilization (Wu et al., 2006) and affect future animal production efficiency. Future studies should also investigate whether these dams' exposure to realimentation after periods of nutrient restriction results in compensatory growth and development of the fetal GIT.

Studies on the effects of maternal nutrition on fetal GIT development have produced contradictory findings about maternal over- and under-nutrition during pregnancy. Using the maternal nutrient restriction model, multiparous beef cows exposed to undernutrition between days 30 and 120 of gestation showed variations in fetal gut development (Meyer et al., 2013). In contrast, Duarte et al. (2013) did not record comparable outcomes in Nellore cows during a comparable gestational window causing us to speculate that species, age/parity, previous nutritional plane, diet type, and other potential metabolic pathways can influence modifications.

Maternal parity in the ruminant model on developmental programming is gaining attention since it has been shown to affect the fetal intestinal development of calves and lambs born to nutrient-restricted mothers (Meyer et al., 2010). Typically, pregnant heifers will require more nutrients to support growth, mammary development, and rapidly growing fetus (Lohakare et al., 2012) and to acquire sufficient body conditions necessary for calving (Abhishek et al., 2018). Considering this, the response to maternal perturbations during pregnancy in heifers compared to mature cows is arguably different.

The combined effects of maternal nutritional stress and stage of pregnancy appear to vary amongst ruminant species. For example, a study on multiparous ewes under nutrient restriction produced lambs with smaller large and small intestine weights, diameters, and mucosal areas (Trahair et al., 1997). Additionally, in the same study, the full establishment of the fetal GIT was visible at parturition.

In contrast, calves born to restricted maternal diets during early gestation showed an increase in intestinal vascularity and jejunal proliferation (Meyer et al., 2010). The reason for this is unclear; however, one theory could be that the gestational length is an important determinant in fetal GIT development regardless of the timing of the nutritional insult or period of realimentation (Dahl et al., 2016). However, despite the species differences and stage of gestation, we can speculate that IUGR neonates are more likely than premature or term newborns to have compromised GIT functions.

The lack of information surrounding how prenatal nutrition can adversely affect fetal GIT development in ruminants must be promptly addressed in future studies. For instance, where Duarte et al. (2013) reported that calves' fetal small intestinal surface area increased in response to feed restriction throughout pregnancy, Caton et al. (2009) found little to no change in the effects of maternal diet on fetal small intestine mass. In a sheep study, lambs from overfed ewes had poorer (gut-mediated) immunoglobulin absorption in the early postnatal hours than lambs born to ewes who were exposed to dietary restriction (Hammer et al., 2011). Collectively, these studies add to the growing body of evidence that maternal nutritional stress during pregnancy on fetal GIT development, capacity and function is still poorly understood and could be multifactorial.

One major identified drawback from most studies on fetal GIT development is the lack of information on the long-term effects of maternal nutritional perturbations on the offspring and whether a particular feeding regime could reverse any postpartum digestive dysfunction in offspring born to mothers exposed to nutritional stress during pregnancy. This knowledge is essential because the evidence in mice showed that total energy restriction during early pregnancy, followed by adequate nutrition for the remainder of gestation, is mainly linked to cardiovascular and metabolic disease risks during adulthood (Van Gronigen Case et al., 2021).

1.5. An evaluation of the effects of maternal over- and undernutrition on fetal GIT development

1.5.1. Maternal undernutrition

A few studies that contrast the effects of overnutrition and undernutrition during pregnancy on fetal GIT development are shown in Table 1.2. Pregnant dams are often subjected to nutrient restriction through either inadequate nutrient intake by grazing poor quality forage and/or intensive management (Meyer et al., 2013) or an attempt to reduce feed cost to improve economic returns could have detrimental impacts on fetal organ development (Serrano-Pérez et al., 2020). Gestating ruminants raised on pasture are very often found to have poor fetal organ development because of the seasonality of the fodder, which will be made worse by periods of maternal nutrient insufficiency in the forage (Costa et al., 2021). This compromise between the requirement for nutrients and the forage availability for dams leads to times of poor nutrition.

For instance, pregnant ewes on pasture in the western USA only receive less than half of the NRC's recommendations (Long et al., 2009; Meyer et al., 2011). The sheep perform poorly during physiological cycles as a result, which negatively affects fetal development and causes adaptive fetal responses (Vautier and Cadaret, 2022). These pasture-raised animals will continue to have poor organ development if feed supplementation is not incorporated into the feeding regimen, especially in tropical locations.

In nutrient-deficient mothers, the fetus sacrifices tissues such as the intestine and muscle to prioritize brain growth (a phenomenon referred to as redistribution of cardiac output), resulting in impaired development of other fetal organs such as the GIT (McMillen et al., 2001; Desai et al., 2005; Fall, 2009). In underfed sheep, fetal brain blood flow was increased, but blood flow to the gastrointestinal tract, kidneys, and peripheral vascular beds was decreased during days 118 and 125 of gestation (Giussani et al., 1993).

Gao et al. (2008) later documented that substrates such as glucose and oxygen are redistributed with the change in fetal cardiac output. Thus, redistribution of fetal cardiac output as an adaptation to chronic maternal malnutrition plays an essential role in maintaining the relative growth and optimal function of major fetal organs (Gao et al., 2008). However, the redistribution of cardiac output away from regional circuits in late gestation may have some negative consequences (McMillen et al., 2001) that could selectively inhibit the growth of GIT tissues and have an impact on cell size (hypertrophy) or cell number (hyperplasia) (Gao et al., 2008). It can be hypothesized that these tissue losses allow the animal to reduce its maintenance requirements and thus increase its chances of survival during the restriction period (Ryan et al. 1993).

The phenomena of prioritizing organ growth in impoverished mothers have earlier been called "selective fetal organ development" using the pig model (Cao et al., 2014). Small intestine weight and weight-length ratio decreased due to maternal undernutrition, and the small intestine's villi were shorter after birth (Cao et al., 2014). Additionally, analysis of small intestinal length after birth revealed that piglets from mothers who were nutritionally deficient had longer small intestines than their peers, indicating that increased relative small intestine absorption area may be a mechanism by which growth-restricted neonates increase their growth rate once they are in an external environment with unlimited access to food (Cao et al., 2014). This information from the pig model is lacking in ruminants as it is important to know if underdeveloped GIT caused by nutritional stress during pregnancy can be improved postnatally with specialized feeds or a feeding program.

The GIT, especially the small intestine, has the ability to adapt to changing physiological demands such as pregnancy (Guilloteau et al., 2009; Meyer and Caton, 2016), stage of gestation (Meyer et al., 2010) or nutrient intake during pregnancy (Ferraris et al., 2001; Carlson et al., 2009). In mice, it was reported that the intestinal weight of mothers whose energy intake was restricted was 12% lower than mothers fed *ad libitum* (Ferraris et al., 2001). Interestingly, the absorption efficiency of the intestine in energy-restricted mice was increased by 60% (Ferraris et al., 2001).

Fetal GIT morphology in response to maternal nutrient restriction is often used to assess the overall impact of developmental programming. For instance, intestinal length, rather than weight, is usually evaluated in fetal programming research as a marker of the correct development of the fetal intestine during gestation or used as a factor for gut adaptability (Weaver et al., 1991) or predictor of adequate intestinal performance (Trahair et al., 1997). Interestingly, intestinal length in nutrient-restricted dams has been reported to increase with advancing gestation, indicating a compensatory response to the low supply of nutrients (Duarte et al., 2013; Meyer and Caton, 2016).

Maternal nutrient restriction throughout gestation can also affect the development of the gut concerning villus length without changing the ultimate length evidenced in fetal bovine (Gionbelli et al., 2016). Regarding digestive capacity, pregnant heifers restricted to 65% of their nutrient requirement all through the first trimester of pregnancy produced daughter calves with decreased milk intake capacity at day 25 post-calving due to less development of the digestive tract *in utero* (Noya et al., 2019). This finding suggests that a prolonged period of nutritional restriction during gestation can affect the digestive functions and capacity of the offspring in postnatal life.

In addition to the effects of maternal nutrient restriction on overall offspring performance, the number of offspring delivered may also affect fetal digestive tract development. Vonnahme et al. (2003) reported higher caruncle vascularity in sheep carrying twins than single lambs. The increased caruncle vascularity may be an adaptive mechanism developed to cope with the increased nutritional requirements of pregnant twins. Future research on twin-carrying ruminants in fetal programming could be an exciting area to expand our knowledge on the interaction between maternal nutrition, gestational stage, and the number of fetuses during gestation on the development of fetal GIT.

1.5.2. *Maternal overnutrition*

Gestating ruminants exposed to overnutrition either through intensive management or excessive intake from lush forages during pregnancy can present a stress level on the dams, which inherently influences fetal GIT development (Meyer et al., 2013). Generally, the main limitation of fetal growth in pregnant mothers occurs independently of high or low concentrations of essential nutrients in the maternal circulation. For example, Zago et al. (2019) reported that an oversupply of energy during pregnancy could limit fetal development, resulting in low-birth-weight calves, while Zhang et al. (2022) concluded that either high or low nutrition during gestation could alter the adipose tissue mass or adipocyte size in fetal cattle.

Similar to undernutrition, the ruminant GIT adapts during the interaction between the gestation window and excessive nutrient intake, as evidenced in ewe studies (Meyer et al., 2013; Yunusova et al., 2013). In both studies, fetal jejunal hypertrophy was impaired when ewes were fed high selenium on day 20 (Meyer et al., 2013) and day 180 (Yunusova et al., 2013) of gestation, and these effects were also evident in adulthood.

Interesting results on the effects of overnutrition on fetal GIT development have also been reported from intestinal data. Earlier development of fetal villi was observed in overfed cows compared to their mid-pregnancy counterparts (Gionbelli et al., 2016). In late gestation, ewes' overfeeding (150% energy and 110% protein) increased and shifted the digestive capacity for carbohydrates toward the jejunum (Khanal et al., 2020). Interestingly, the prenatal effects of overnutrition were most pronounced in the duodenum, where the fetal villous enlargement factor was increased (Khanal et al., 2020). However, the reduced villous thickness was observed with increased insulin receptor substrate-1 (IRS -1) and reduced growth hormone receptor (GH-R) expression (Khanal et al., 2020).

Evidence in cows (Duarte et al., 2013) and sheep (Yanusova et al., 2013), showed impaired fetal intestinal development when pregnant dams were overfed (> maintenance level). However, postnatal observations from the sheep study showed that lambs from overfed ewes (140% of nutrient requirement) had higher intestinal maltase activity than their counterparts (Yanusova et al., 2013), suggesting an adaptive response to the excessive nutrient environment during the prenatal period (Vautier and Cadaret, 2022).

Multiple studies have provided evidence of developmental programming of the digestive enzymes in ruminants fed either nutrient-restricted diets or 100% compliance with NRC recommendations. Because digestive enzymes respond to changes in nutrient intake (Kreikemeier et al. 1990), it is imperative to investigate whether enzymatic activities could be impaired during nutritional programming in ruminants.

In ewes, Trotta et al. (2020) reported that fetal small intestinal maltase, glucoamylase, and isomaltase activity were absent in dams fed a 40% restricted diet between first and mid-pregnancy. In cattle, reduced fetal pancreatic trypsin activity was observed in dams-fed nutrient-restricted diets (60% of nutrient requirements) during late gestation (Keomanivong et al., 2017a). Yanusova et al. (2013) found no differences in fetal jejunal maltase activity at day 180 of pregnancy between ewes fed 60%, 100%, and 140% of nutrient requirements. The species and gestation stage differences observed in these studies should be considered as an important variable in building a ruminant model to show whether nutrient restriction or 100% NRC compliance can be used for programming purposes.

Although there are discrepancies in the responses of fetal digestive enzymes in ruminants to prenatal nutrition, these highlighted studies in this review demonstrate the potential of programming fetal digestive enzymes in ruminants.

1.6. Systems perspective of maternofetal nutritional programming in a dairy cow

Systems thinking has been used for many decades for helping to understand how critical variables are interconnected in solving many different types of problems (Senge, 1990; Tedeschi et al., 2023). However, it has become even more so as the systems we rely on have become more complex and interrelated. System thinkers sketch their understanding of the problem through causal loop diagrams. A general picture of maternofetal nutritional programming in a named ruminant can be presented (Figures 3a and 3b). By explaining the system and emphasizing the feedback loops that connect the most crucial variables and components, these strategies enable one to raise the complexity of understanding in order to predict potential future behaviors and impacts. Currently, causal diagrams are used in the agriculture and food systems to identify sustainable relationships and recommend alternate approaches (Molina Benavides et al., 2017; Correddu et al., 2020).

Figures 3a and 3b summarizes 4-four loops identified as drivers of fetal programming. Systems thinking and analysis are often used to qualitatively analyze interactions among system elements using causal maps or causal loop diagrams. R1 loop has a positive reinforcing effect between maternal nutrient supply and the growing fetus. The more the dairy industry and farmers are aware of the importance of proper nutritional management during pregnancy, the better the uptake of nutrients by the mothers during pregnancy which will improve not only maternal growth, condition and health during pregnancy, however, in turn, provide more nutrients that are available to the fetus for both cellular and tissue development. This agrees with several studies conducted using maternal nutrient restriction models or IUGR approach (Trahair et al., 1997; Du et al., 2010, Costa et al., 2021).

Balancing loops B1, B2 and B3 show balancing effects of fetus growth, intake limit and lactation performance, respectively (Figure 3a). Fetal growth can offset (or impair) the same fetal organ development (B1) if the mother restricts nutrient supply. Comparative study has shown that the normal and expected fetal organ growth and development is achieved if fetal body growth is not impaired (Trahair et al., 1997). Likewise, limiting maternal intake/nutrition (B2) would potentially limit fetal nutrient requirements, and thus, result in an inherent negative maternal nutrient balance.

As previously reported, there is competition between mother and fetus for the limited available nutrients, therefore selection of organ development is prioritized (McCoski et al., 2021). This has the potential to cause permanent changes in behavior, metabolism, milk production performance, and create welfare concerns later in life (B3). This effect can propagate across generations and affect overall herd performance (Figure 3b). This is consistent with a study in late gestation heat stressed cows, which found that progenies born to mothers who were subjected to heat stress 46 days before calving had lower milk production in their first lactation cycle (Laporta et al., 2020).

It is worth noting that the systems thinking representation of developmental or fetal programming in cows discussed in this review did not consider the timing of maternal nutritional insult. Nonetheless, the findings of the various studies evaluated in this review show that altering maternal nutrition during pregnancy can affect fetal GIT development at any stage of pregnancy.

1.7. CONCLUSION

To conclude, one functional and important parameter for healthy development *in utero* is that the GIT must have a sufficient surface area and capacity for digestion and nutrition absorption when enteral or postnatal feeding begins. The capacity and function of the GIT after birth must therefore be understood in relation to the development of the fetus at each stage of pregnancy.

Perturbations in maternal nutrition throughout different stages of pregnancy is a key driver of fetal GIT programming in ruminants. However, there are some contradictions in the literature regarding the precise effects of nutritional disruption on fetal GIT development, and more study is required to close these knowledge gaps because while maternal undernutrition during gestation is chiefly associated with IUGR, maternal overnutrition is capable in resetting the development of the fetal GIT organs in ruminants. In addition, we are unsure as to whether postnatal nutritional schedules exist in ruminants to correct any potentially defective GIT development that may have occurred *in utero.*

Currently, it is impossible to define exact strategies for nutrient supplementation in each gestational window for proper GIT development because fetal organ growth could be impaired as early as in the first trimester of pregnancy. In addition, there is still a gap in knowledge when dams are exposed to environmental factors (such as heat stress, overcrowding, pollutants, or transport) or welfare on fetal GIT development and how these factors may predispose multigenerational effects in offspring is still not known. This remains a major challenge for future research because it is likely that each of these elements will have an impact on fetal GIT development at the same time. This suggests that certain detrimental consequences of prenatal programming may be due only to dietary stress, while others may be connected to other environmental stresses.

Because a bulk of the studies on fetal GIT programming have stopped at the fetal life, there is a need for further studies to investigate the long-term or/ multigenerational impact of compromised fetal GIT and, thus, identify faster interventional management strategies in compromised offspring to ensure practical GIT usage in the future life span of production animals and for general herd productivity.

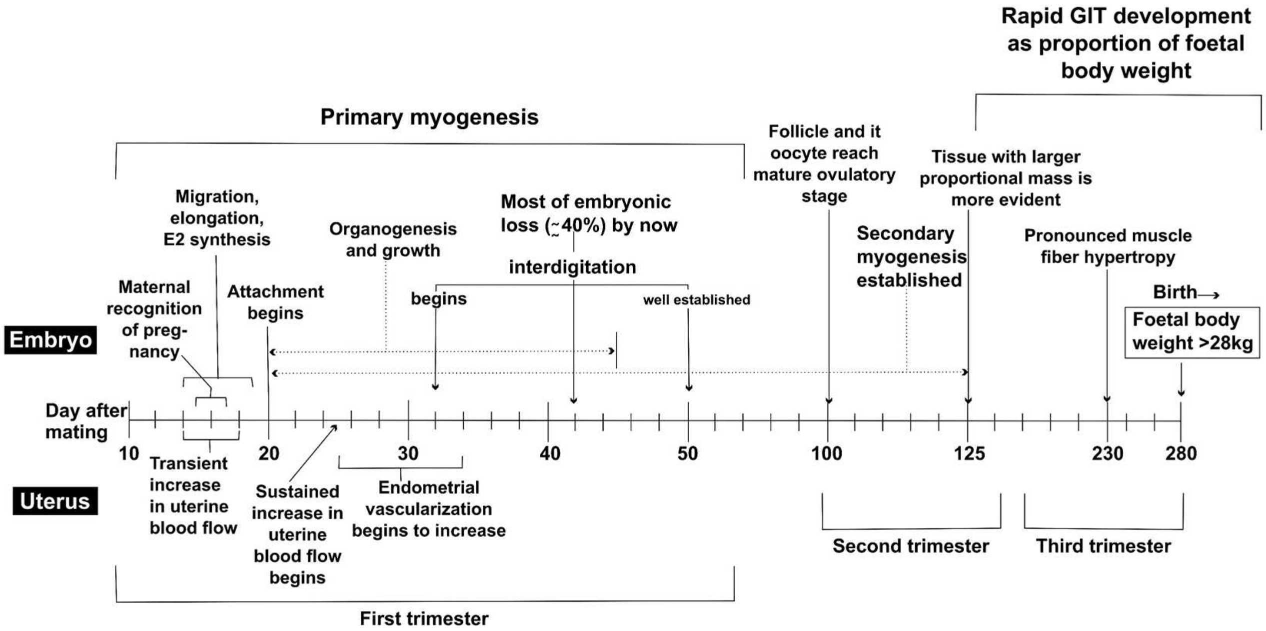


Figure 1 Schematic representation of the development of the placenta and the fetus throughout gestation in cattle. Time between conception and day 50 of pregnancy was modified from Caton et al. 2020 (with permission). Information on myogenesis and muscle fibre hypertrophy were obtained from Du et al. 2010 while fetal body weight between day 269 and birth was obtained from Duarte et al. 2013. As illustrated, organogenesis of most organs including the GIT is well established at d 50 after mating. Although at d 125, tissues with larger proportional mass are more evident, there is accelerated growth of these organs in late gestation.

Table 1.1 Timeline of the commencement of fetal GIT development in ruminants and related species

References	Specie	*Day of gestation
Warner, 1958	Bovine	56
Guilloteau et al., 2009	Cattle	30
Vivo and Robina, 1991	Cow	30
García, et al., 2012	Goat	35
Ward et al., 2006	Sheep	40-65
David et al., 2010	Sheep	55
Rodrigues et al., 2014	Horse	25
Sase et al., 2001	Rabbit	24
Everaert et al., 2017	Swine	~45
Masot et al., 2007	Red deer	60
Montgomery et al., 1999	Human	30

* The day of gestation here refers to the actual moment the authors were able to visualize the appearance of the fetal GIT.

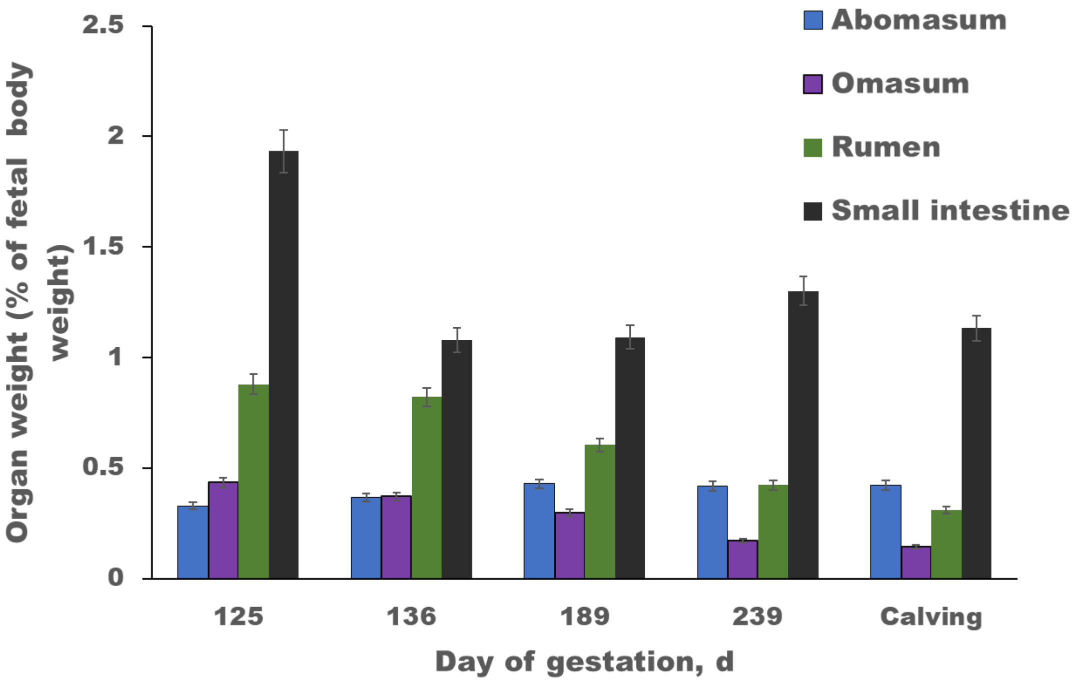


Figure 1.2. Weight of the forestomach and small intestine of single female fetal cattle from early gestation to calving. Fetuses were from mothers of different genetic origins, different environmental conditions, and different maternal diets. Organ weights were expressed as % of fetal live body weight. Data was compiled from experiments conducted by Becker et al., 1951, Meyer et al., 2010 & Duarte et al., 2013

Table 1.2. Selected studies on maternal over - versus under nutrition on ruminant fetal GIT development during different stages of gestation.

Reference	Specie	Methodology	Gestation (day)	Observations
Meyer et al., 2010	Beef cows	CON (12.1% CP, 70.7% IVDMD, DM basis) Vs RES (9.9% CP, 54.5% IVDMD, DM basis)	30-245	↑ GIT, Omasum and stomach complex in CON
Khanal et al., 2020	Sheep	Low (50% energy and protein) Vs High (150% energy and protein)	Last trimester	↑ Lactate gene expression in High
Meyer et al., 2013	Sheep	60% (Res) Vs 140% (High) nutrient requirement	40 – term	↔ Small intestinal mass
Trahair et al., 1997	Sheep	High (>60kg) +Clover grass-hay Vs Res (15kg) of nutritional intake	0-90	↑ Total fetal GIT wt in High fed lambs
Scheaffer et al., 2004	Sheep	CON (100%) Vs. RES (60%) dietary intake	50-130	↑ Small intestinal mass in RES ↑ Capillary area density in RES

Table 1.2. Continued

Reference	Specie	Methodology	Gestation (day)	Observations
Trotta et al., 2020	Sheep	CON (100% nutrient req.) Vs RES (60% nutrient req.)	54 – term	↔ Starch digestive enzymes
Zhang et al., 2021	Cow	120% Vs 60% nutritional requirement	0 - 260±8	↑ Rumen, omasum, and large intestine in CON ↔ Abomasum and small intestine
Prezotto et al., 2014	Sheep	60% RES Vs. 100% CON	50-130	↓ Liver and Jejunum mass in RES
Duarte et al., 2013	Nellore cattle	Ad libitum Vs 1.2 times maintenance diet	47-269	↑ Surface area of small intestine in RES ↔ Total fetal GIT, rumen, omasum, abomasum and small intestinal length
Peine et al., 2018	Sheep	CON (100% nutrient requirement) Vs RES – ARG (60% + 180 mg/kg BW ARG supplement)	54 – term	↔ Total GIT, large and small intestinal weight at day 54 post-natal

RES: Nutrient restricted; CON: Control (Adequate per NRC recommendations; ARG: Arginine; IVDMD: in-vitro dry matter digestibility, ↔ = no significant effect, wt = weight ↑ = increase ↓ = decrease

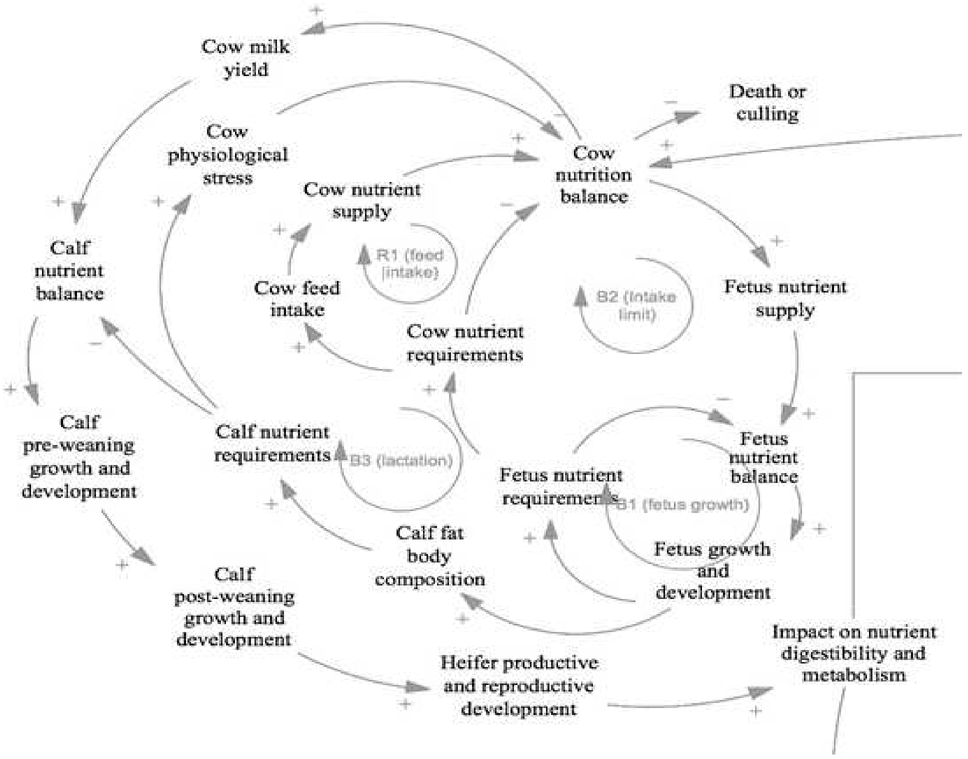


Figure 1.3a. Conceptual model of mother metabolism on fetal programming and herd performances. Arrows indicate causality, whereas polarity signs, + and -, indicate positive and negative correlation among variables, B (B1=fetus growth, B2=intake limit, B3=lactation) and R (R1=feed intake) indicates balancing and reinforcing loops, respectively (Sterman, 2000).

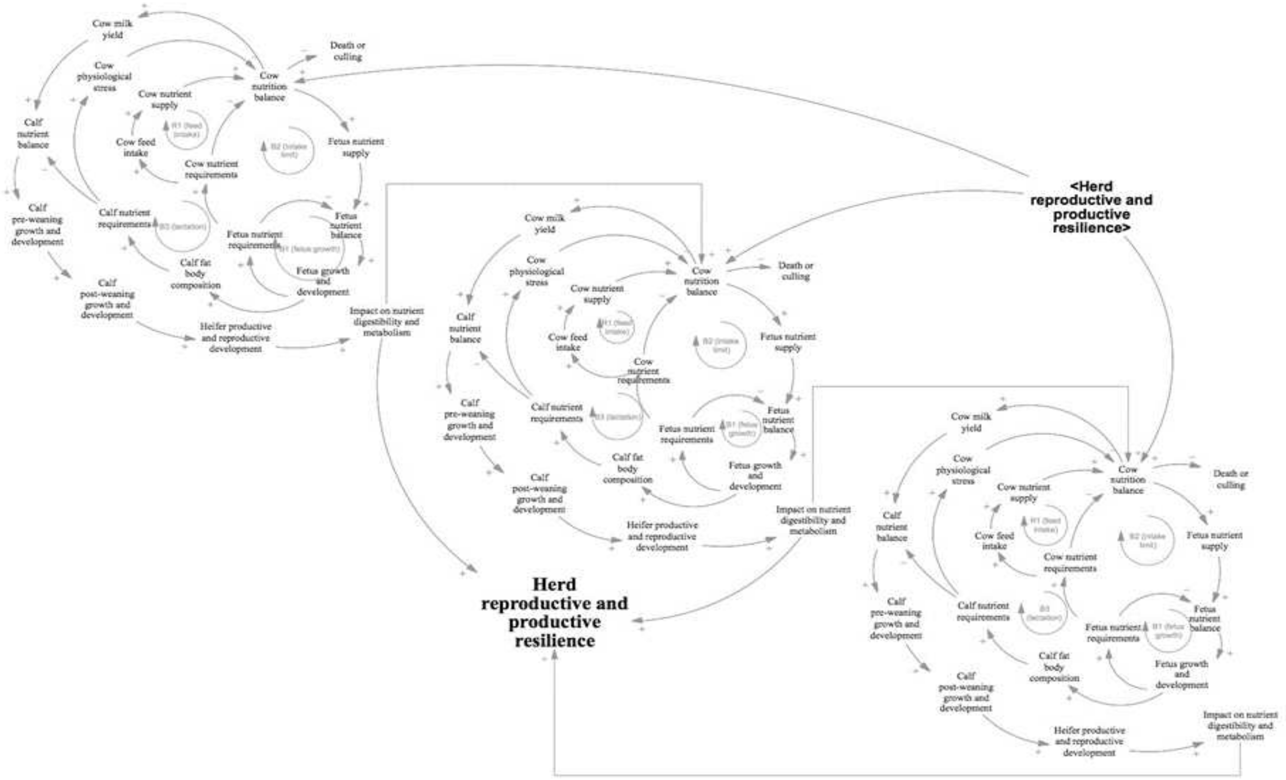


Figure 1.3b. Propagation of the dynamics presented in Figure 1.3a through generations and feedback on the herd.

REFERENCES

Abhishek, P., Bhakat, C., Mondal, S., Mandal, D.K. and Ghosh, P.R. 2018. Body condition score is not a predictor of back fat in primiparous crossbred cattle. Int. J. Basic Appl. Biol. 5(2018): 45-47. https://doi.org/10.31220/osf.io/q5ndf.

Aguila, M.B., Ornellas, F. and Mandarim-de-Lacerda, C. A. 2021. Nutritional Research and Fetal Programming: Parental Nutrition Influences the Structure and Function of the Organs. International Journal of Morphology. 39(1): http://dx.doi.org/10.4067/S0717-95022021000100327.

Al Masri, S., Reincke, R., Huenigen, H., Gemeinhardt, O., Richardson, K.C. and Plendl, J. 2018. Computed tomography study of the fetal development of the dairy cow stomach complex. J. Dairy Sci. 1719–1729. https://doi.org/10.3168/jds.2017-13508.

Avila, C., Harding, G., Rees, R. and Robinson, S. 1989. Small intestinal development in growth-retarded fetal sheep. J. Pediatr. Gastroenterol. Nutr. *8*: 507-515. https://doi:10.1097/00005176-198905000-00015.

Baldwin, R.L., McLeod, K.R., Klotz, J.L. and Heitmann, R.N. 2004. Rumen Development, Intestinal Growth and Hepatic Metabolism in the Pre- and Postweaning Ruminant. J. Dairy Sci. 87(2004): E55-E65. https://doi.org/10.3168/jds.S0022-0302(04)70061-2.

Barker, D.J.P. 1990. Fetal and infant origins of adult disease. London, BMJ Publishing Group. https://doi.org/10.1136/bmj.301.6761.1111.

Barker, D.J.P. 2004. The developmental origins of adult disease. J. Am. Coll. Nutr. 23 (2004):588S95S. https://doi.org/10.1080/07315724.2004.10719428.

Becker, R., Arnold, P.D. and Marshall, S.P. 1951. Development of the bovine stomach during fetal life. J. Dairy Sci. 34(1951): 329–332. https://doi.org/10.3168/jds.S0022-0302(51)91715-8.

Burgstaller, J., Wittek, T. and Smith, G.W. 2017. Invited review: Abomasal emptying in calves and its potential influence on gastrointestinal disease. J. Dairy Sci. 100(2017): pp.17-35. https://doi.org/10.3168/jds.2016-10949.

Cao, M.M.A., Lianqiang, C., Wang, J.M.A., Yang, M.M.A., Guoqi-Su, M.A., Zhengfeng, F., Yan, L., Shengyu, X. and Wu, D. 2014. Effects of maternal over- and undernutrition on intestinal morphology, enzyme activity, and gene expression of nutrient transporters in newborn and weaned pigs. Nutrition, 30(2014):1442–1447. https://doi.org/10.1016/j.nut.2014.04.016.

Carlson, D.B., Reed, J.J., Borowicz, P.P., Taylor, J.B., Reynolds, L.P., Neville, T.L., Redmer, D.A., Vonnahme, K.A. and Caton, J.S. 2009. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes. J. Anim. Sci. 87(2009):669–680. https://doi.org/10.2527/jas.2007-0837.

Caton, J.S. and Hess, B.W. 2010. Maternal plane of nutrition: impacts on fetal outcomes and postnatal offspring responses. In: Hess BW, DelCurto T, Bowman JGP, Waterman RC, eds. Proceedings of the 4th Grazing Livestock Nutrition Conference; 2010 Jul 9–10; Champaign (IL). Western Section American Society of Animal Science; (2010):104–22.

Caton, J.S., Bauer, M.L. and Hidari, H. 2000. Metabolic components of energy expenditure in growing beef cattle. Asian-Aus. J. Anim. Sci. 13(2000): 702–710. https://doi.org/10.5713/ajas.2000.702.

Caton, J.S., Crouse, M.S., McLean, K.J., Dahlen, C.R., Ward, A.K., Cushman, R.A., Grazul-Bilska, A.T., Neville, B.W., Borowicz, P.P. and Reynolds, L.P. 2020. Maternal periconceptual nutrition, early pregnancy, and developmental outcomes in beef cattle. J Anim Sci. 2020. 1;98(12): skaa358.

Caton, J.S., Crouse, M.S., McLean, K.J., Dahlen, C.R., Ward, A.K., Cushman, R.A., Grazul-Bilska, A.T., Neville, B.W., Borowicz, P.P. and Reynolds, L.P. 2020. Maternal periconceptual nutrition, early pregnancy, and developmental outcomes in beef cattle. J Anim Sci. 1;98(12):skaa358. doi: 10.1093/jas/skaa358.

Caton, J.S., Crouse, M.S., Reynolds, L.P., Neville, T.L., Dahlen, C.R., Ward, A.K., and Swanson, K.C. 2019. Maternal nutrition and programming of offspring energy requirements. Transl. Anim. Sci. 3(2019), 976-990. https://doi.org/10.1093/tas/txy127.

Caton, J.S., Reed, J.J., Aitken, R., Milne, J.S., Borowicz, P.P., Reynolds, L.P., Redmer, D.A. and Wallace, J.M. 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(2009): 222–235. https://doi.org/10.2527/jas.2008-1043.

Chu, D.M., Ma, J., Prince, A., Antony, K.M., Seferovic, M.D. and Aagaard, K.M. 2017. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat. Med. 23(2017): 314–326. https://doi.org/10.1038/nm.4272.

Chu, D.M., Meyer, K.M., Prince, A.L. and Aagaard, K.M. 2016. Impact of maternal nutrition in pregnancy and lactation on offspring gut microbial composition and function. Gut Microbes. 7(2016): 459-470. https://doi.org/10.1080/19490976.2016.1241357.

Clifton, V.L., Moss, T.J., Wooldridge, A.L., Gatford, K.L., Liravi, B., Kim, D., Muhlhausler, B.S., Morrison, J.L., Davies, A., De Matteo, R., Wallace, M.J. and Bischof, R.J. 2017. Development of an experimental model of maternal allergic asthma during pregnancy. J. Phys. 594(2017):1311–1325. https://doi.org/10.1113/JP270752.

Connor, E.E., Li, R.W., Baldwin, R.L. and Li, C. 2010. Gene expression in the digestive tissues of ruminants under their relationships with feeding and digestive processes. Animals, 4(2010): pp. 993-1007. https://doi.org/10.1017/S1751731109991285.

Correddu, F., Lunesu, M.F., Buffa, G., Atzori, A.S., Nudda, A., Battacone, G., and Pulina, G. 2020. Can Agro-Industrial By-Products Rich in Polyphenols be Advantageously Used in the Feeding and Nutrition of Dairy Small Ruminants? Animals (Basel), 10:131. https://doi.org/10.3390/ani10010131.

Costa, T.C., Du, M., Nascimento, K.B., Galvão, M.C., Meneses, J.A.M., Schultz, E.B., Gionbelli, M.P. and Duarte, M.D.S. 2021. Skeletal Muscle Development in Postnatal Beef Cattle Resulting from Maternal Protein Restriction during Mid-Gestation. Animals (Basel) 11(2021): 860. https://doi.org/10.3390/ani11030860.

Couture, A. 2008. Fetal gastrointestinal tract: US and MR. In: Couture A, Baud C, Ferran FL et al (eds) Gastrointestinal tract sonography in fetuses and children. Springer, Berlin Heidelberg, 2008, pp. 1-84.

Cronjé, P.B. 2003. Foetal programming of immune competence. Aust. J. Exp. Agri. 43(003): pp. 1427-1430. https://doi.org/10.1071/EA03005.

David, A.L., Abi-Nader, K.N., Weisz, B., Shaw, S.W.S., Themis, M., Cook, T., Coutelle, C., Rodeck, C.H. and Peebles, D.M. 2010. Ultrasonographic development of the fetal sheep stomach and evaluation of early gestation ultrasound-guided *in utero* intragastric injection. Taiwan J. Obstet. Gynecol. 49(2010). https://doi.org/10.1016/S1028-4559(10)60004-6.

Desai, M., Gayle, D., Babu, J. and Ross, M.G. 2005. Permanent reduction in heart and kidney organ growth in offspring of undernourished rat dams. Amer. J. Obstet. Gynaecol. 193(2005): 1224–32. https://doi.org/10.1016/j.ajog.2005.05.041.

Diao, Q., Zhang, R. and Fu, T. 2019. Review of Strategies to Promote Rumen Development in Calves. Animals. 9(2019): 490. https://doi.org/10.3390/ani9080490.

Dobson, D.E., Prager, E.M. and Wilson, A.C. 1984. Stomach lysozymes of ruminants. Distribution and Catalytic Properties. J. Biol. Chem. 250(1984):11607-11616. https://doi.org/10.1016/S0021-9258(18)90907-5.

Du, M., Ford, S.P. and Zhu, M.J. 2017. Optimizing livestock production efficiency through maternal nutritional management and fetal developmental programming. Animal Frontiers, 7(2017): 5–11. https://doi.org/10.2527/af.2017-0122.

Du, M., Tong, J., Zhao, J., Underwood, K.R., Zhu, M., Ford, S.P. and Nathanielsz, P.W. 2010a. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88(2010a): E51–E60. https://doi.org/10.2527/jas.2009-2311.

Duarte, M.D.S., Gionbelli, M.P., Paulino, P.V.R., Seraoc, N.V.L., Martins, T.S., Totaro, P.I.S., Neves, C.A., Filho, S.C.V., Dodson, M.V., Zhu, M. and Du, M. 2013. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. Livest. Sci. 153(2013): pp. 60-65. https://doi.org/10.1016/j.livsci.2013.01.006.

Echternkamp, S.E. 1993. Relationship between placental development and calf birth weight in beef-cattle. Anim. Reprod. Sci. 32(1993):1-13. https://doi.org/10.1016/0378-4320(93)90053-T.

Everaert, N., Van Cruchten, S., Weström, B., Bailey, M., Van Ginneken, C., Thymann, T. and Pieper, R. 2017. A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis. Anim. Feed Sci. Techn. 233(2017): 89–103. https://doi:10.1016/j.anifeedsci.2017.06.011

Fall, C. 2009. Maternal nutrition: effects on health in the next generation. Indian J. Med. Res. 130(2009): pp. 593–9. https://link.gale.com/apps/doc/A229721118/HRCA?u=googlescholar&sid=googleScholar&xid=20389997.

Ferraris, R.P., Cao, Q.X., and Prabhakaram, S. 2001. Chronic but not acute energy restriction increases intestinal nutrient transport in mice. J. Nutr. 131(2001): 779-786. https://doi.org/10.1093/jn/131.3.779.

Fleming, T.P., Kwong, W.Y., Porter, R., Ursell, E., Fesenko, I., Wilkins, A., Miller, D.J., Watkins, A.J. and Eckert, J.J. 2004. The embryo and its future. Biol Reprod. 71(2004): pp. 1046-54. https://doi.org/10.1095/biolreprod.104.030957.

Fowden, A.L., Giussani, D.A., and Forehead, A.J. 2006. Intrauterine programming of physiological systems causes and consequences. Physiology (Bethesda). 21(2006):29-37. https://doi.org/10.1152/physiol.00050.2005.

Fraga, F.J.R., Lagisz, M., Nakagawa, S., Villalobos, N.L., Blair, H.T. and Kenyon, P.R. 2018. Meta-analysis of lamb birth weight as influenced by pregnancy nutrition of multiparous ewes. J. Anim. Sci. 96(2018):1962–1977. https://doi.org/10.1093/jas/sky072.

Fubini, S.L. and Ducharme, N.G. 2004. Chapter 10 - Surgery of the Bovine Digestive System. Editor(s): Susan L. Fubini, Norm G. Ducharme. Farm Animal Surgery. W.B. Saunders, 2004, pp. 161-281. https://doi.org/10.1016/B0-72-169062-9/50014-9.

Funston, R.N., Larson, D.M. and Vonnahme, K.A. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. J. Anim. Sci. 88(E.Suppl. 2010): E205- E215. https://doi.org/10.2527/jas.2009-2351.

Gaebel, G., Aschenbach, J.R. and Müller, F. 2001. Transfer of energy substrates across the ruminal epithelium: implications and limitations. Anim. Health Res. Rev. 3(2001):15-30. https://doi.org/10.1079/AHRR200237.

Gao, F., Hou, X. and Liu, Y. 2008. Effect of intrauterine growth restriction on weight and cellularity of gastrointestinal tract in postnatal lambs. Can. J. Anim. Sci. 88(2008):107-112. https://doi.org/10.4141/CJAS07115.

García, A., Masot, J., Franco, A., Gázquez, A. and Redondo, E. 2012. Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. Anat. Rec. (Hoboken), 295(2012): 776-785. https://doi.org/10.1002/ar.22431.

Gardner, D.S., Buttery, P.J., Daniel, Z. and Symonds, M.E. 2007. Factors affecting birth weight in sheep: maternal environment. Reproduction. 133(2007): 297-307. https://rep.bioscientifica.com/downloadpdf/journals/rep/133/1/1330297.xml.

Gartner, H., Shukla, P., Markesich, D.C., Solomon, N.S., Oesterreicher, T.J. and Henning, S.J. 2002. Developmental expression of trehalase: role of transcriptional activation. Biochim. Biophys. Acta. 1574(2002): 329-36. https://doi.org/10.1016/S0167-4781(02)00231-2.

Gilbert, J.S., Ford, S.P., Lang, A.L., Pahl, L.R., Drumhiller, M.C., Babcock, S.A., Nathanielsz, P.W., and Nijland, M.J. 2007. Nutrient restriction impairs nephrogenesis in a gender specific manner in the ovine fetus. Paediat. Res. 61(2007): 42-47. https://doi.org/10.1203/01.pdr.0000250208.09874.91.

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

Giussani, D.A., Spencer, J.A.D., Moore, P.J., Bennet, L. and Hanson, M.A. 1993. Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. J. Physiol. (Lond.) 461(1993): 431-449. https://doi.org/10.1113/jphysiol.1993.sp019521.

Godfrey, K.M. and Barker, D.J. 2000. Fetal nutrition and adult disease. Amer. J. Clin. Nutr. 71(Suppl.2000): 1344S–1352S. https://doi.org/10.1093/ajcn/71.5.1344s.

Greenwood, P.L. and Bell, A.W. 2003. Consequences of intra-uterine growth retardation for postnatal growth, metabolism and pathophysiology. Reproduction. Suppl. 61(2003):195-206. https://doi.org/10.1530/biosciprocs.5.015.

Greenwood, P.L. and Café, L.M. 2007. Café Prenatal and pre-weaning growth and nutrition of cattle: long-term consequences for beef production. Animal, 1(2007):1283–129. https://doi.org/10.1017/S175173110700050X.

Gruenwald, P. 1963. Chronic fetal distress and placental insufficiency. Biology of Neonate. 5(1963): 215–232. https://doi.org/10.1159/000239870.

Guilloteau, P., Zabielski, R. and Blum, J.W. 2009. Gastrointestinal tract and digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulation. J. Physiol. Pharm. 60 (Suppl.3 2009): 37-46. https://pubmed.ncbi.nlm.nih.gov/19996480/.

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

Han, H.C., Austin, K.J., Nathanielsz, P.W., Ford, S.P., Nijland, M.J. and Hansen, T.R. 2004. Maternal nutrient restriction alters gene expression in the ovine fetal heart. J. Physiol. 558(2004): 111-121. https://doi.org/10.1113/jphysiol.2004.061697.

Harding, J.E. and Johnston, B.M. 1995. Johnston Nutrition and fetal growth. Reproduction, Fertililty and Development. 7(1995): 539–547. https://doi.org/10.1071/RD9950539.

Hofmman, P.C. 2017. Feeding management of the dairy heifer from 4 months to calving. In: Beede DK, (Ed.), Large Dairy Herd Management. Champaign: American Dairy Science Association. P. 431-444.

Hubbert, W.T., Stalheim, O.H.V. and Booth, G.D. 1972. Changes in organ weights and fluid volumes during growth of the bovine fetus. Growth 36(1972): 217–233. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL7336108223.

Jennings, T.D., Gonda, M.G., Underwood, K.R., Wertz-Lutza, A.E. and Blair, A.D. 2015. The influence of maternal nutrition on expression of genes responsible for adipogenesis and myogenesis in the bovine fetus. Animals. 10(2015):1697–1705. https://doi:10.1017/S1751731116000665.

Kenyon, P.R. and Blair, H.T. 2014. Foetal programming in sheep - Effects on production. Small Rumin. Res. 118(2014): 16-30. https://doi.org/10.1016/j.smallrumres.2013.12.021.

Keomanivong, F.E., Camacho, L.E., Lemley, C.O., Kuemper, E.A., Yunusova, R.D., Borowicz, P.P., Kirsch, J.D., Vonnahme, K.A., Caton, J.S. and Swanson, K. 2017a. Effects of realimentation after nutrient restriction during mid- to late gestation on pancreatic digestive enzymes, serum insulin and glucose levels, and insulin-containing cell cluster morphology. J. Anim. Physiol. Anim. Nutr. (Berl.). 101(2017a): 589 – 604. https://doi:10.1111/jpn.12480.

Khan, M.A., Weary, D.M., and von Keyserlingk, M.A.G. 2011. Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. J. Dairy Sci. 94(2011):1071-1081. https://doi.org/10.3168/jds.2010-3733

Khanal, P.D., Axel, A.M., Safayi, S., Elbrønd, V.S. and Nielsen, M.O. 2020. Prenatal over- and undernutrition differentially program small intestinal growth, angiogenesis, absorptive capacity, and endocrine function in sheep. Physiol Rep. 8(2020): e14498. https://doi:10.14814/phy2.14498.

Kreikemeier, K.K., Harmon, D.L., Peters, J.P., Gross, K.L., Armendariz, C.K., and Krehbiel, C.R. 1990. Influence of dietary forage and feed intake on carbohydrase activities and small intestinal morphology of calves. J. Anim. Sci. 68(1990):2916–2929. https://doi:10.2527/1990.6892916x.

Langley-Evans, S.C. and McMullen, S. 2010. Developmental origins of adult disease. Medical Principles and Practice. 19(2010): 87–98. https://doi.org/10.1159/000273066.

Limesand, S.W., Rozance, P.J., Zerbe, G.O., Hutton, J.C. and Hay Jr, W.W. 2006. Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. Endocrinology 147(2006): 1488–1497. https://doi.org/10.1210/en.2005-0900.

Liu, K., Zhang, Y., Yu, Z., Xu, Q., Zheng, N., Zhao, S., Huang, G. and Wang, J. 2021. Ruminal microbiota–host interaction and its effect on nutrient metabolism. Anim. Nutr. 7(2021): pp. 49-55. https://doi.org/10.1016/j.aninu.2020.12.001.

Lohakare, J.D., Südekum, K.H. and Pattanaik, A.K. 2012. Nutrition-induced Changes of Growth from Birth to First Calving and Its Impact on Mammary Development and First-lactation Milk Yield in Dairy Heifers: A Review. Asian-Austral. J. Anim. Sci. 25(2012):1338-50. https://doi.org/10.5713/ajas.2012.12282.

Long, N.M., Vonnahme, K.A., Hess, B.W., Nathanielsz, P.W. and Ford, S.P. 2009. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. J. Anim. Sci. 87(2009): 1950–1959. https://doi:10.2527/jas.2008-1672.

Lu, V.B., Gribble, F.M. and Reimann, F. 2021. Nutrient-Induced Cellular Mechanisms of Gut Hormone Secretion. Nutrients. 13(2021):883. https://doi.org/10.3390/nu13030883.

Lunesu, M.F., Ledda, A., Correddu, F., Fancello, F., Marzano, A., Mossa, F., Nudda, A., Cannas, A. and Atzori, A.S. 2020. Prenatal exposure to different diets influences programming of glucose and insulin metabolism in dairy ewes. J. Dairy Sci. https://doi.org/10.3168/jds.2020-18342.

Martin, J.L., Vonnahme, K.A., Adams, D.C., Lardy, G.P. and Funston, R.N. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. J. Anim. Sci. 85(2007): 841-847. https://doi.org/10.2527/jas.2006-337.

Masot, A.J., Franco, A.J. and Redondo, E. 2007. Morphometric and immunohistochemical study of the abomasum of red deer during prenatal development. J. Anat. 211(2007): 376–386. https://doi:10.1111/j.1469-7580.2007.00772.x.

McLeod, K.R. and Baldwin, R.L. 2000. Effects of diet forage: Concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. J. Anim. Sci. 78(2000): 760–770. https://doi.org/10.2527/2000.783760x.

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

Meale, S.J., Durand, F.C., Berends, H., LuoGuan, L. and Steele, M.A. 2017. From pre- to postweaning: Transformation of the young calf's gastrointestinal tract. J. Dairy Sci. 100(2017): 5984-5995. https://doi.org/10.3168/jds.2016-12474.

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

Meyer, A.M., Neville, T.L., Reed, J.J., Taylor, J.B., Reynolds, L.P., Redmer, D.A., Hammer, C.J., Vonnahme, K.A. and Caton, J.S. 2013. Maternal nutritional plane and selenium supply during gestation impact visceral organ mass and intestinal growth and vascularity of neonatal lamb offspring. J. Anim. Sci. 91(2013): 2628–2639. https://doi.org/10.2527/jas.2012-5953.

Meyer, A.M., Reed, J.J., Neville, T.L., Thorson, J.F., Maddock-Carlin, K.R., Taylor, J.B., Reynolds, L.P., Redmer, D.A., Luther, J.S., Hammer, C.J., Vonnahme, K.A. and Caton, J.S. 2011. Nutritional plane and selenium supply during gestation affect yield and nutrient composition of colostrum and milk in primiparous ewes. J. Anim. Sci. 89(2011): 1627–1639. https://doi.org/10.2527/jas.2010-3394.

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

Micke, G.C., Sullivan, T.M., Soares Magalhaes, R.J., Rolls, P.J., Norman, S.T. and Perry, V.E.A. 2010. Heifer nutrition during early- and mid-pregnancy alters fetal growth trajectory and birth weight. Anim. Reprod. Sci. 117(2010): 1-10. https://doi.org/10.1016/j.anireprosci.2009.03.010.

Molina Benavides, R.A., Sánchez Guerrero, H., Campos Gaona R, Atzori, A.S. and Morales, J.D. 2017. Dynamic estimation of greenhouse gas emissions from bovine livestock of Valle del Cauca, Colombia. Acta Agronómica. 66(2017): 422–429. https://doi.org/10.15446/acag.v66n3.58266.

Montgomery, R.K., Mulberg, A.E. and Grand, R.J. 1999. Development of the Human Gastrointestinal Tract: Twenty Years of Progress. Special reports and reviews, Gastroenterology. 116(1999): 702–731. https://doi.org/10.1016/S0016-5085(99)70193-9.

Mossa, F., Bebbere, D., Ledda, A., Burrai, G.P., Chebli, I., Antuofermo, E., Ledda, S., Cannas, A., Fancello, F. and Atzori, A.S. 2018. Testicular development in male lambs prenatally exposed to a high-starch diet. Molecular Reproductive Development. 85(2018): 406–416. https:/ /doi .org/10.1002/mrd .22974.

Mulvihill, S.J., Albert, A., Synn, A. and Fonkalsrud, E.W. 1985. In utero supplemental fetal feeding in an animal model: effects on fetal growth and development. Surgery 98 (1985): 500–505. https://pubmed.ncbi.nlm.nih.gov/4035570/.

Myatt, L. 2006. Placenta adaptive responses and fetal programming. J. Physiol. 25-30. https://doi.org/10.1113/jphysiol.2006.104968.

Nagata, A., Hatta, S., Ji, X., Ishikawa, A., Sakamoto, R., Yamada, S., Imai, H., Matsuda, T. and Takakuwa, T. 2019. Return of the intestinal loop to the abdominal coelom after physiological umbilical herniation in the early fetal period. J. Anat. 234(2019): 456-464. https://doi.org/10.1111/joa.12940.

Nathanielsz, P.W. 2006. Nathanielsz Animal models that elucidate basic principles of the development origins of adult diseases. Institute of Laboratory Animal Research. J47:73-82. https://doi.org/10.1093/ilar.47.1.73.

Noya, A., Casasús, I., Ferrer, J. and Sanz, A. 2019. Long-Term Effects of Maternal Subnutrition in Early Pregnancy on Cow-Calf Performance, Immunological and Physiological Profiles during the Next Lactation. Animal, 9(2019): 936. https://doi:10.3390/ani9110936.

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

Paliy, O., Piyathilake, C.J., Kozyrskyj, A., Celep, G., Marotta, F. and Rastmanesh, R. 2014. Excess body weight during pregnancy and offspring obesity: Potential mechanisms. Nutrition 30(2014):245–251. https://doi.org/10.1016/j.nut .2013.05.011.

Peine, J.L., Jia, G., Van Emon, M.L., Neville, T.L., Kirsch, J.D., Hammer, C.J., O'Rourke, S.T., Reynolds, L.P. and Caton, J.S. 2018. Effects of maternal nutrition and rumen-protected arginine supplementation on ewe performance and postnatal lamb growth and internal organ mass. J. Anim. Sci. 98(2018): 3471-3481. https://doi.org/10.1093/jas/sky221.

Peterson, L.W. and Artis, D. 2014. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat. Rev. Immunol. 14(2014):141-53. https://doi.org/10.1038/nri3608.

Pitkin, R.M. and Reynolds, W.A. 1975. Fetal ingestion and metabolism of amniotic fluid protein. Amer. J. Obstet. Gynecol. 123(1975): 356-63. https://doi.org/10.1016/S0002-9378(16)33436-6.

Prezotto, L.D., Lemley, C.O., Camacho, L.E., Doscher, F.E., Meyer, A.M., Caton, J.S., Awda, B.J., Vonnahme, K.A. and Swanson, K.C. 2014. Effects of nutrient restriction and melatonin supplementation on maternal and foetal hepatic and small intestinal energy utilization. J Anim. Physiol. Anim. Nutr. (Berl). 2014 (4):797-807. doi: 10.1111/jpn.12142.

Rae, M.T., Kyle, C.E., Miller, D.W., Hammond, A.J., Brooks, A.N. and Rhind, S.M. 2002. The effects of undernutrition, in utero, on reproductive function in adult male and female sheep. Anim. Reprod. Sci. 72(2002): 63-71. https://doi.org/10.1016/S0378-4320(02)00068-4.

Ravelli, A.C., Bleker, O.P., Roseboom, T.J., Van Montfrans, G.A., Osmond, C. and Barker, D.J.P 2005. Cardiovascular disease in survivors of the Dutch famine. Nestle Nutr Workshop Ser Pediatr Program 55: 183–91; discussion 191–5. https://doi.org/10.1159/000082602.

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

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

Reeves, P. G., Nielsen, F. H. and Fahey Jr., G. C. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. Journal of Nutrition. 123(11):1939-51.

Reynolds, L.P., Borowicz, P.P., Caton, J.S., Vonnahme, K.A., Luther, J.S., Hammer, C.J., Maddock, K.R., Carlin, A.T. Grazul-Bilska and Redmer, D.A. 2020. Developmental programming: The concept, large animal models, and the key role of uteroplacental vascular development. Journal of Animal Science. 88(2010): E61–E72. https://doi.org/10.2527/jas.2009-2359.

Rodrigues, M.N., Carvalho, R.C., Franciolli, A.L.R., Rodrigues, R.F., Rigoglio, N.N., Jacob, J.C.F., Gastal, E.F. and Miglino, M.A. 2014. Prenatal Development of the Digestive System in the Horse. The Anatomical Records. 297(2014):1218-1227. https://doi.org/10.1002/ar.22929.

Ross, M.G and Nijland, M.J. 1998. Development of ingestive behavior. Amer. J. Physiol. 274(1998): R879-93. https://doi.org/10.1152/ajpregu.1998.274.4.R879.

Ryan, W.J., Williams, I.H. and Moir, R.J. 1993. Compensatory growth in sheep and cattle. II. Changes in body composition and tissue weights. Austr. J. Agricul. Res. 44: 1623-1633. https://doi.org/10.1071/AR9931609.

Sangild, P.T., Fowden, A.L. and Trahair, J.F. 2000. How does the foetal gastrointestinal tract develop in preparation for enteral nutrition after birth? Livest. Prod. Sci. 66(2000):141–150. https://doi:10.1016/S0301-6226(00)00221-9.

Sangild, P.T., Petersen, Y.M., Schmidt, M., Elnif, J., Petersen, T.K., Buddington, R.K., Greisen, G., Michaelsen, K.F. and Burrin, D.G. 2002. Preterm birth affects the intestinal response to parenteral and enteral nutrition in newborn pigs. J. Nutri. 132(2002):3786-94. https://doi:10.1093/jn/132.9.2673.

Sase, M., Lee, J.J., Park, J.Y., Thakur, A., Ross, M.G. and Buchmiller-Crair, T.L. 2001. Ontogeny of fetal rabbit upper gastrointestinal motility. J. Surg. Res. 101(2001):68-72. https://doi:10.1006/jsre.2001.6254.

Scheaffer, A.N., Caton, J.S., Redmer, D.A., Arnold, D.R. and Reynolds, L.P. 2004. Effect of dietary restriction, pregnancy, and fetal type on intestinal cellularity and vascularity in Columbia and Romanov ewes. J. Anim. Sci. 82(2004): 3024–3033. https://doi.org/10.2527/2004.82103024x.

Serrano-Pérez, B., Molina, E., Noya, A., López-Helguera, I., Casasús, I., Sanz, A., and Villalba, D. 2020. Maternal nutrient restriction in early pregnancy increases the risk of late embryo loss despite no effects on peri-implantation interferon-stimulated genes in suckler beef cattle. Research in Veterinary Science. 128(2020): 69-75. https://doi.org/10.1016/j.rvsc.2019.10.023.

Shen, Z.M., Seyfert, H.M., Lohrke, B., Schneider, F., Zitnan, R., Chudy, A., Kuhla, S., Hammon, H.M., Blum, J.W., Martens, H., Hagemeister, H. and Voigt, H. 2004. An energy-rich diet causes rumen papillae proliferation associated with more IGF type I receptors and increased plasma IGF-I concentrations in young goats. J. Nutr. 134(2004):11–17. https://doi.org/10.1093/jn/134.1.11.

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

Stalker, L.A., Adams, D.C., Klopfenstein, T.F., Feuz, D.M. and Funston, R.N. 2006. Effects of pre- and postpartum nutrition on reproduction in spring calving cows and calf feedlot performance. J. Anim.Sci. 84(2006): 2582-2589. https://doi.org/10.2527/jas.2005-640.

Sterman, J.D. 2000. Business Dynamics. Systems Thinking and Modelling for a Complex World. Irwin McGraw-Hill: Boston. https://www.researchgate.net/publication/44827001_Business_Dynamics_System_Thinking_and_Modeling_for_a_Complex_World.

Summers, A.F. and Funston, R.N. 2013. Fetal Programming: Implications for Beef Cattle Production. Proc. The Range Beef Cow Symposium XXIII. Rapid City, SD. Pp 29- 40.

Sun, Z.H., He, Z.X., Zhang, Q.L., Tan, Z.L., Han, X.F., Tang, S.X., Zhou, C.S., Wang, M. and Yan, Q.X. 2013. Effects of energy and protein restriction, followed by nutritional recovery on morphological development of the gastrointestinal tract of weaned kids. J. Anim. Sci. 91(2013): 4336–4344. https://doi.org/10.2527/jas.2011-4500.

Tedeschi, L.O., Adams, J. and Mendonca Vieira, R.A. 2023. Revisiting Mechanisms, Methods, and Models for Altering Forage Cell Wall Utilization for Ruminants. Journal of Animal Science. DOI: 10.1093/jas/skad009.

Tomoda, S., Brace, R.A. and Longo, L.D. 1985. Amniotic fluid volume and fetal swallowing rate in sheep. Amer. J. Physiol. 249(1985): R133-R138. https://doi.org/10.1152/ajpregu.1985.249.1.R133.

Trahair, J.F. and Sangild, P.T. 2002. Sangild Studying the devel.opment of the small intestine: philosophical and anatomical perspectives. In: Zabielski R, Gregory PC, Westrom B, eds. Biology of the intestine in growing animals. Amsterdam: Elsevier Science; 2002.1–54. https://www.researchgate.net/publication/251472010_Chapter_1_Studying_the_development_of_the_small_intestine_philosophical_and_anatomical_perspectives.

Trahair, J.F., DeBarro, T.M., Robinson, J.S., Julie, A. and Owens, J.A.1997. Restriction of Nutrition in Utero Selectively Inhibits Gastrointestinal Growth in Fetal Sheep. J. Nutr. 127(1997): 637–641. https://doi.org/10.1093/jn/127.4.637.

Trotta, R.J. and Swanson, K.C. 2021. Prenatal and Postnatal Nutrition Influence Pancreatic and Intestinal Carbohydrase Activities of Ruminants. Animals, 11(2021):171. https://doi.org/10.3390/ ani11010171.

Trotta, R.J., Keomanivong, F.E., Peine, J.L., Caton, J.S. and Swanson, K.C. 2020. Influence of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activity of lamb offspring. Livest Sci. 241:104246. https://doi.org/10.1016/j.livsci.2020.104246.

Underwood, M.A. and Sherman, M.P. 2006. Nutritional Characteristics of Amniotic Fluid. NeoReviews 7 (2006): e310-e316. https://doi.org/10.1542/neo.7-6-e310.

USDA, 2007. Part 1: Reference of dairy cattle health and management practices in the United States, 2007. NAHMS Dairy, pp. 1-128.

Van der Meer, Y., Lammers, A., Jansman, A.J., Rijnen, M.M., Hendriks, W.H. and Gerrits, W.J. 2016. Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. J. Anim. Sci. 94(2016):4704-4719. https://doi.org/10.2527/jas.2016-0787.

Van Gronigen Case, G., Storey, K.M., Parmeley, L.E. and Schulz, L.C. 2021. Effects of maternal nutrient restriction during the periconceptional period on placental development in the mouse. PLoS ONE 16(2021): e0244971. https://doi.org/10.1371/journal.pone.0244971.

Vautier, A.N. and Cadaret, C.N. 2022. Long-Term Consequences of Adaptive Fetal Programming in Ruminant Livestock. Front. Anim. Sci. 3(2022):778440. https://doi.org/10.3389/fanim.2022.778440.

Vivo, J.M. and Robina, A. 1991. The development of the bovine stomach: morphologic and morphometric analysis. II. Observations of the morphogenesis associated with the omasum and abomasum. Anatomy and Histological Embryology. 20(1991):10–17. https://doi:10.1111/j.1439-0264.1991.tb00286.x.

Vonnahme, K.A. 2012. Vonnahme How the maternal environment impacts fetal and placental development: Implications for livestock production. Anim. Reprod. 9(2012):789–797. http://cbra.org.br/pages/publicacoes/animalreproduction/issues/download/v9n4/p789-797.pdf.

Vonnahme, K.A., Hess, B.W., Hansen, T.R., McCormick, R.J., Rule, D.C., Moss, G.E., Murdoch, W.J., Nijland, M.J., Skinner, D.C., Nathanielsz, P.W. and Ford, S.P. 2003. Maternal undernutrition from early- to mid-gestation leads to growth retardation, cardiac ventricular hypertrophy, and increased liver weight in the fetal sheep. Bio. Reprod. 69(2003):133-140. https://doi.org/10.1095/biolreprod.102.012120.

Wang, K.C.W., Zhang, L., McMillen, I.C., Botting, K.J., Duffield, J.A., Zhang, S., Suter, C.A., Brooks, D.A. and Morrison, J.L. 2011. Fetal growth restriction and the programming of heart growth and cardiac insulin-like growth factor 2 expression in the lamb. J. Physiol. 589(2011): 4709–4722. https://doi.org/10.1113/jphysiol.2011.211185.

Ward, V.L., Estroff, J.A., Nguyen, H.T., Lakshmanan, Y., Hayward, A., Zurakowski, J.D., Dunning, P.S., Peters, C.A. and Barnewolt, C.E. 2006. Fetal sheep development on ultrasound and magnetic resonance imaging: A standard for the in-utero assessment of models of congenital abnormalities. Fetal Diagnosis and Therapy, 21(2006):444-457. https://doi.org/10.1159/000093888.

Warner, E.D. 1958. The organogenesis and early histogenesis of the bovine stomach. Amer. J. Anat. 102(1958):33. https://doi.org/10.1002/aja.1001020103.

Warner, R.G., Flatt, W.P. and Loosli, J.K. 1956. Dietary factors influencing the development of the ruminant stomach. Agriculture and Food Chemistry. 4(1956):788-792. https://doi.org/10.1021/jf60067a003.

Watson, R.R. and McMurray, D.N. 1979. McMurray The effects of malnutrition on secretory and cellular immune processes. Crit. Rev. Food Sci. Nutr. 12(1979):113-59. https://doi.org/10.1080/10408397909527275.

Weaver, L.T., Austin, S. and Cole, T.J. 1991. Small intestinal length: a factor essential for gut adaptation. Gut, 32(1991): 1321-1323.https://gut.bmj.com/content/gutjnl/32/11/1321.full.pdf.

Windeyer, M.C., Lesliea, K.E., Godden, S.M., Hodgins, D.C., Lissemorea, K.D. and LeBlanc, S.J. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Preventive Veterinary Medicine. 113(2014):231–240. https://doi.org/10.1016/j.prevetmed.2013.10.019.

Wu, G., Bazer, F.W., Cudd, T.A., Meininger, C.J. and Spencer, T.E. 2004. Maternal nutrition and fetal development. J. Nutr. 134(2004): 2169-2172. https://doi.org/10.1093/jn/134.9.2169.

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

Xu, R.J., Mellor, D.J., Birtles, M.J., Reynolds, G.W. and Simpson, H.V. 1994. Impact of intrauterine growth retardation on the gastrointestinal tract and the pancreas in newborn pigs. J. Pediat. Gastroenterol. Nutrition. 18(1994): 231-240. https://doi:10.1097/00005176-199402000-00018.

Yunusova, R.D., Neville, T.L., Vonnahme, K.A., Hammer, C.J., Reed, J.J., Taylor, J.B., Redmer, D.A., Reynolds, L.P. and Caton, J.S. 2013. Impacts of maternal selenium supply and nutritional plane on visceral tissues and intestinal biology in 180-day-old offspring in sheep. J. Anim. Sci. 91(2013): 2229–2242. https://doi.org/10.2527/jas.2012-5134.

Zago, D., Canozzi, M.E.A. and Barcellos, J.O.J. 2019. Pregnant cow nutrition and its effects on foetal weight–a meta-analysis. J. Agricul. Sci. 157(2019):83–95. https://doi.org/10.1017/S0021859619000315.

Zhang, Y., Otomaru, K., Oshima, K., Goto, Y., Oshima, I., Muroya, S., Sano, M., Roh, S. and Gotoh, T. 2022. Maternal Nutrition During Gestation Alters Histochemical Properties, and mRNA and microRNA Expression in Adipose Tissue of Wagyu Fetuses. Front. Endocrinol. (Lausanne). 1(2022): 797680. https://doi: 10.3389/fendo.2021.797680.

CHAPTER 2

Objectives

GENERAL OBJECTIVES

This thesis was conceived from a previous study (Mossa et al., 2013) that investigated maternal undernutrition in cross bred heifers during early gestation, where impaired ovarian and cardiovascular systems in their progenies was observed. Comparative studies have attempted to examine the gastrointestinal system of offspring whose mothers' nutrition during pregnancy was altered.

Herein, the primary objective of this work was to investigate the effects of a feeding program (maternal undernutrition and overnutrition) and day of gestation in heifers from shortly before conception to early gestation on the development and function of the gastrointestinal tract (GIT) in their offspring. The specific objectives were:

1. Investigating the effects of maternal energy restriction or overnutrition in dairy heifers during the first 80 and 120 d of gestation on the growth performance, dry matter intake (DMI), apparent total tract digestibility and rumination behavior in the second and third trimesters of pregnancy.
2. Evaluating the effect of day of gestation on apparent total tract digestibility in pregnant heifers.
3. Comparing the growth performance, starter intake, apparent nutrient digestibility and rumination behavior of pre-weaned calves born to mothers exposed to early gestation nutrient restriction or overnutrition.
4. Evaluating post weaning calves' responses to DMI, growth performance, behavior, apparent total tract digestibility, visceral and gastrointestinal organs weight, small and large intestinal length and digesta particle size distribution along the GIT.

Three studies from the primary hypothesis were conducted to achieve these objectives:

- Chapter 3: Nutrient restriction in heifers during early pregnancy: effects on maternal growth performance, apparent total tract digestibility, intake, and voluntary behaviour in the subsequent trimesters and on calf early body measurements.
- Chapter 4: This chapter studied the effects of early-gestation maternal energy restriction in heifers on solid feed intake, behavioural patterns, growth performance and apparent total tract digestibility in pre-weaned dairy calves.
- Chapter 5: Here, we studied early-gestation maternal energy restriction in dairy heifers on gastrointestinal development, organ weight, behaviour and digesta particle size distribution in post weaned dairy calves.

The specific hypothesis being tested is that progenies exposed to maternal undernutrition in early gestation will have underdeveloped gastrointestinal tract at post weaning.

CHAPTER 3

Nutrient restriction in heifers during early pregnancy: effects on maternal growth performance, apparent total tract digestibility, intake, and voluntary behaviour in the subsequent trimesters and on calf early body measurements

ABSTRACT

The effects of a feeding program in early pregnancy on growth performance, apparent total tract digestibility, DMI, and behaviour of heifers in the second and third trimesters of pregnancy, and on calf birth body measurements was investigated. Based on body weight and age, Holstein Friesian heifers (n=28; initial BW±SD; 371.57±42.14) were assigned to three experimental groups to receive: i) 0.6 of their maintenance energy requirements (M) until day 80 of gestation (nutrient restricted NR80, n = 11), ii) day 120 of pregnancy (NR120, n=11) and iii) 1.8M (Control, n=6) until day 120 of pregnancy. Starting 11 days before artificial insemination, heifers were individually fed the assigned TMR diet with the same energy and protein content. Heifers were group fed once the individual feeding period ended, with *ad libitum* access to feed until calving. Body weight was measured monthly during gestation while calves' body measurements were recorded at birth and throughout the first 6 weeks of postnatal. All dependent maternal and calf variables studied were analyzed as mixed models with repeated measures in R. Growth performance among the three feeding groups was significantly ($P<0.001$) dependent on day of gestation and nutritional status of the heifer in early gestation. Daily gains all through gestation were significantly different ($P<0.0001$) among the experimental groups with heifers in the nutrient restricted groups having higher gains when exposed to refeeding until late gestation. Maternal BCS was significantly higher in the control fed heifers than their counterparts ($P<0.001$). However, at both calving and 30 days post parturition, BCS was similar for all heifers. Independent of the experimental group, heifers BCS significantly decreased after parturition ($P<0.0001$). The low DMI (~4.5 kg/day) in early gestation improved significantly ($P<0.05$) in the nutrient restricted groups as gestation progressed and was comparable to the values in the Control group at d 230 of gestation. In late gestation, time spent feeding was similar for heifers in the NR120 (343 min/day) and Control (327 min/day) groups, but significantly lower ($P<0.05$) for heifers in the NR80 (303 min/day) group. Crude protein, dry matter and NDF apparent total tract digestibility was significantly better in NR80 fed heifers than their counterparts. However, as gestation progressed, apparent total tract digestibility was similar across the experimental groups. Rumination time below the minimum threshold (<390 min/day) in nutrient restricted groups early in gestation improved significantly (NR80=638, NR120=614 min/day) and was higher in late gestation than in the Control group (590 min/day). Body weight of calves at birth was significantly different ($P=0.017$) among all groups with calves from control fed heifers heavier than those from NR80, while control calves tended to be heavier than NR120 calves. The feeding program and duration of feeding in early gestation had no effect on gestational length and calves ADG. Overall, an interaction between feeding program and gestation day for all maternal dependent variables studied was detected. Collectively, these results suggest that pregnant heifers fed restricted energy diets early in gestation can compensate for nutrient losses later in gestation, regardless of the duration of restricted feeding. It is recommended that the implications of these results for future heifer and calf performance be further investigated.

Key words: Early gestation, nutrient restriction, behaviour, intake, digestibility

3.1 INTRODUCTION

The historical assumption that higher nutrient requirements, particularly higher energy requirements, do not occur until mid- to late gestation and that cow nutrition cannot affect calf growth until the last trimester of gestation (NRC, 2001; NASEM, 2016; Alharthi et al., 2021) remains one of the current challenges facing the rearing and nutritional management of replacement heifers. Even if this assumption is correct, the common practice of feeding total mixed ration (TMR) refusals from lactating cows to growing heifers (Boterman and Bucholtz, 2005) or forage seasonality in extensive reared ruminants (Costa et al., 2022) among other factors, further complicates the nutritional management of these animals during pregnancy. However, current findings contradict the above theory, as there is evidence that the relationship between calf postnatal survival and their general performance depends on the energetic nutritional status of the individual in the fetal environment, especially in early gestation (Duarte et al., 2013, Noya et al., 2019).

Considering that limit feeding is one of the few nutrient restriction strategies used with great success in ruminants, this feeding impairs dry matter intake, resulting in lower weight gain (Paradis et al., 2017) and body condition score (Hoffman et al., 2007), alters rumination (Heinrichs et al., 2021) and feeding behavior (Greter et al., 2011), and exposes animals to numerous hazards. For example, cattle that are fed less exhibit stereotyped oral behavior (Redbo and Nordblad, 1997; Lindstroem and Redbo, 2000), an increased tendency to reach inaccessible feed (Williams et al., 2022), prolonged vocalization (Hoffman et al., 2007; Tucker et al., 2008), and increased inactive standing time (Hoffman et al., 2007; Greter et al., 2011). These activities pose a welfare concern because, for example, foot disease is associated with prolonged standing in lactating cows (Ramanoon et al., 2018) and behavioural changes such as increased vocalization indicate hunger and frustration (Greter et al., 2011; Franchi et al., 2019) and lack of satiety (Savory et al., 1993).

Most data on behaviours in pregnant cattle have been published in late gestation because they are useful as key indicators, one of which is predicting the onset of calving in dairy cows (Miedema et al., 2011a; Pahl et al., 2014). Severe behavioural changes in cows have been observed mostly on the day of calving compared to the days before calving (Pahl et al., 2014; Braun et al., 2014; Barraclough et al., 2019; Matamala et al., 2021). For example, 4 days prior to calving, primiparous cows laid down an average of 2.8 h/d less and walked more steps/d compared to multiparous cows (Barraclough et al., 2019). On the day of calving, Schirmann et al. (2013) reported a 15% reduction in rumination, a 24% reduction in DMI, and a 32% reduction in time spent eating compared to 2 to 4 days prior to calving.

Several studies indicate that pregnancy can reduce dry matter intake (Schirmann et al., 2013; Linden et al., 2014; Pérez-Báez et al., 2019) and DM apparent total tract digestibility in dairy cows (Rotta et al., 2015; Pereira et al., 2020) and that reduced DMI is exacerbated in late pregnancy (French, 2006, Rotta et al., 2015). The reduction in DMI in late gestation could be explained by the rapid decrease in feed intake due to the increase in volume of the gravid uterus (occupying approximately 60% of the stomach) (Hummel et al., 2021), which affects rumen volume (Forbes, 2009) and thus reduces the mean retention time of digestive products (Reynolds et al., 2004). In Holstein x Gyr cows, cows fed *ad libitum* showed a significant decrease in DMI (~40%) as gestation progressed between gestation d 150 and 262 compared to the group fed at the limit (1.15% of body weight) (Rotta et al., 2015). In terms of DMI as a % of body weight, Pereira et al. (2020) reported a decrease in DMI between day 200 and 250 of gestation in grazing Holstein x Gyr cows, and the severity of this change can lead to negative energy balance prior to calving (Dann et al., 1999).

Despite these findings, it is important to investigate in heifers whether DMI or DMI as a percentage of body weight declines at a comparable rate in late gestation as in adult cows because these heifers are still growing. In this study, it is speculated that nutrient restricted gravid heifers in the first trimester of gestation will eat continuously from the feed bunk during realimentation and regain body condition in late gestation, refuting the existing theory of DMI reduction in late gestation.

Less is known regarding the duration of restricted feeding during early pregnancy might affect DMI, apparent total-tract digestibility, growth performance, or voluntary behavioural activities of heifers in subsequent trimesters. Long et al. (2012) attempted to examine growth patterns of heifers during pregnancy and found that limit fed heifers (55% of NRC recommendations) during 32 to 115 days of gestation lost body weight (-63 kg) and BCS (4.3 vs. 5.5) prior to calving compared to heifers fed 100% of NRC recommendations. In the same study, gestation length was shorter in heifers receiving low nutrient intakes from day 32 to day 115 of gestation (274 vs. 278) than in heifers receiving moderate nutrient intakes.

The objective of this study was to investigate the effects of a feeding program and day of gestation on DMI, maternal behavioural activities, apparent tract digestibility, growth performance and calf body measurements at birth. The hypothesis being tested is that nutrient-restricted dairy heifers in early gestation, with reduced maintenance energy requirement, would have better apparent total tract digestibility and would compensate for nutrient losses in the later stages of gestation, as evidenced by compensatory growth, improved DMI, rumination time, shorter gestation length, and similar calf weight at birth.

3.2 MATERIALS AND METHODS

The experiment was conducted in a commercial dairy farm in Northern Sardinia (Mores, Italy; 40°33'0 "N, 8°50'0 "E). All experimental animals were handled in accordance with DPR 27/1/1992 (Animal Protection Regulations of Italy) in conformity with European Community regulation 86/609 and were approved by the local Committee for the Animal Welfare of the University of Sassari, Italy (Prot.n0001848 of 2/5/2019).

3.2.1. Animals, Experimental design, and Management

Our experimental design is summarized in Figure 3.1. Holstein Friesian dairy heifers (n = 42) between 14 and 17 months of age and with a mean initial body weight (BW± SD; 366.2±41.1) were randomly selected and used in this study from April 2021 to March 2022. Heifers were assigned to one of three experimental groups [nutrient restricted: (NR80, n = 15; NR120, n = 17;) or overfeeding: Control, n = 10)] and were balanced for age and weight. To mimic the previous feeding program by Mossa et al. (2013), all heifers were individually fed a dry total mixed ration (TMR) 11 days prior to the start of breeding that was either 1.8 or 0.6 M of their maintenance energy (M) requirements. Generally, pregnant heifers weighing 300kg are fed between 1-1.2 M (intake Mcal/d ~ 15.2 or DMI ~6.77 kg/d).

Heifers in the Control received 1.8 M during the first 120 days of gestation, while heifers in the nutrient restricted [NR80 and NR120] groups received 0.6 M in the first 80 and 120 days of gestation, respectively.

3.2.2 Estrus synchronization protocol and artificial insemination

On the first day of differential feeding regime, the reproductive tract of the heifers was examined by transrectal ultrasound (MyLab^{TM}Omega, Esaote with a 7.5 MHz linear probe) by the farm vet. Heifers with a corpus luteum (CL) received two prostaglandin (PG) treatments administered 10 days apart (Cloprostenol, PGFVeyx^{TM}, Bayer; 2ml IM).

Following the gynaecological examination, an individual heat synchronization protocol for the 42 selected heifers was identified based on the individual cycle phase, thus allowing artificial insemination (AI) to be performed on all the heifers in the same day. The synchronization provided the administration of a double dose of prostaglandin (PGFVeyx^{TM}, Bayer; 2ml SC) after 10 days for the heifers who presented a corpus luteum at the second gynaecological visit. In heifers without CL and which, on the other hand, at the time of the gynaecological examination had only follicles >10 mm in diameter received Gonadotropin Releasing Hormone, small follicles with no corpus luteum, GnRH (Gonadorelin, Enagon^{TM}, Intervet productions; 2ml IM) was administered in order to promote ovulation and the formation of the corpus luteum, followed by an injection of prostaglandin (PGFVeyx^{TM}, Bayer; 2ml I/M) after 10 days. Starting 12h after the second injection, heifers were visually monitored to detect signs of estrus and the presence of a preovulatory follicle was confirmed by ultrasonography.

Approximately 8h after heat, heifers expected to be pregnant with a single female calf were artificially inseminated with sex-sorted frozen-thawed semen from a single sire (Barbaro, In Seme), in order to minimize the variables in the experimental trial. In this study, the day of insemination was considered as day 0 of pregnancy. Sex-sorted semen was used to increase the number of female calves born, and as it was already used and positively evaluated in various in vitro tests. Furthermore, it was positively evaluated genetically for the elevated CR % (Conception rate: the number of pregnant cows divided by the number of inseminated cows).

3.2.3. Monitoring of pregnancy diagnosis and fetal sex assessment

Following the artificial insemination of the heifers, ultrasound examinations were scheduled for diagnosis and confirmation of pregnancy. Pregnancy was diagnosed and monitored Gynaecological examinations were performed with the help of the veterinarian gynaecologist, using an ultrasonography ultrasound device (MyLab^{TM}Omega, Esaote) connected to a linear rectal 7,5 MHz probe).

Diagnosis was made starting from 28 day of gestation post-AI; non-pregnant heifers were excluded from the trial and returned to the herd (Table 3.1). Positive signs of pregnancy were the CL presence (an echogenic, ovoid-shaped structure in the ovary), anechogenic fluid in the ipsilateral uterine horn. The pregnancy diagnosis was performed on 40 animals that responded to heat synchronization, exhibited estrus behavior, and were finally inseminated.

Final pregnancy confirmation was carried out at d 55-60 of gestation, and at this point of growth the fetus has normally reached 6-7 cm in length measured from the head to the lower part of the body (CRL or crown- rump length, CRL), some organs such as the omasum and abomasum and the ossification centres of the skull and vertebrae begin to be visible. It is also possible to perform fetal sexing at this stage.

Fetal sexing was carried out with the identification based on the localization of the genital tubercle; an anatomical structure that is first visible around begins to show around day 41-44 of gestation. Both in the male and female fetuses, the genital tubercle has the sameis ultrasonographic characteristics: bilobed, hyperechoic, and brilliant. Around d 58 of gestation, the tubercle reaches its final position, just caudal to the navel in the male and under the tail in the female. In the male fetus after d 65, the genital tubercle assumes a quadrilobed shape following the formation of the urogenital folds; the external genitals (scrotum and foreskin) will be visible from d 70 of gestation. In the female fetus, the genital tubercle (which will give rise to the clitoris) appears bilobed and the mammary papillae appear; in the period between 80-130 days of gestation the diameter of the mammary glands was 0.6-3 mm.

On d 28 and 55 of gestation (Table 3.1), heifer pregnancy was confirmed by transrectal ultrasound (MyLabOneVet, Esaote, Genoa, Italy). Based on the pregnancy confirmation, the number of animals in an experimental group was determined as [NR80; n = 11; NR120; n = 11; Control; n = 6] and followed until calving. Three pregnant heifers from the nutrient restricted group and one pregnant heifer from the control were confirmed to carry single male fetuses.

During the first 80 and 120 days of gestation, traditional ruminant feeding was performed twice daily at 08:00 and 16:00. Orts from Control were collected and weighed daily to measure individual DMI by subtracting TMR refusals from supplied. Ort data from heifers in NR80 and NR120 were not available because DMI intake (mean ± se) for these groups was 4.34 ± 0.01 and 4.38 ± 0.01/day, respectively. The ration calculated based on altering maintenance energy requirements remained unchanged throughout the individual feeding phase. All heifers received an additional 1 kg of hay per day, particularly to reduce stereotypic behaviour especially in the nutrient restricted groups.

Heifers were group fed once the individual feeding period ended, with *ad libitum* access to feed (1.4M) until calving (Mossa et al., 2013). The wet and dried TMR diets (Table 3.2) used for this experiment were not prepared to be isocaloric or isonitrogenous and were prepared using the large ruminant nutrition system (LRNS). Unlike during the individual feeding phase, heifers were fed once a day with *ad libitum* access to feed all day in the second and third trimesters of pregnancy.

Diet samples were used to determine particle size distribution on an as-fed basis using the Penn State Particle Separator (Lammers et al., 1996). Diet peNDF was calculated as the product of the total diet NDF content and its physical effectiveness factor (Mertens, 1997).

DMI at the end of each individual feeding period was calculated based on predicted equations (NRC, 2001; Hoffman et al., 2008) below:

1. $DMI=12.91\times(1-e(-0.00295\times BW)$ [(Hoffman et al. (2008); end of each individual feeding to day 250 of gestation]
2. $DMI = (1.71-(0.69\times e\text{\^{}}((0.35\times DP-280))))/100\times BW$ [NRC, 2001; >250 to calving]

where e is the Euler number ($e = 2.718$), BW is the body weight and DP is the day in pregnancy.

3.3. Heifer body linear measurements

Body weight, hip height, and hearth girth were measured on days -59, 12, 42, 70, 120, 140, 170, 200, 230, and 265 after they fasted overnight for approximately 7 hours. To carefully monitor weight changes, body weight was measured every two weeks with a tape measure. Hip height was measured with measuring sticks across the hip bones while pregnant heifers were held in a standing position in the chute (Linden et al., 2014).

3.4. Calves body measurement

Three weeks prior to the expected calving date, the heifers were moved to the calving pens with straw bedding and access to water. Each pen had been set up on purpose in an area that was easily accessible to personnel, in order to promote facilitate assistance during calving and timeliness in the first calf care. All 28 single calves born to heifers used in this study were separated immediately from their mothers while body weights and other linear body measurements were recorded at birth. Four out of the 28 calves were males and were excluded from the post calving measurements. One calf died 3 weeks after birth and was excluded from the trial. One calf in the NR80 group had an ovarian pathology and was also excluded from all postnatal measurements.

Body dimension measurements were recorded every fortnight, while maintaining the solid and milk feeding protocol of the commercial farm. Colostrum was administered within the first 6 hours of birth and for the first 4 consecutive days of post-natal life before being introduced to starter and milk replacer (MR). Starter was made available all day, while MR was fed twice daily at 6 L/d via nipple buckets. As with their mothers, calves were made to fast overnight before being weighed the next day.

3.5. Apparent total-tract digestibility and Fecal measurements

Fecal measurements were performed according to the procedure described by Luan et al. (2016). Fecal samples (approximately 400g, wet weight) were collected from the rectum of each heifer into plastic containers at 6 time points (TP) for 3 consecutive days: TP1, d 1, 0800 h; TP2, d 1, 1600 h; TP3, d 2, 0900 h; TP4, d 2, 17:30 h; TP5, d 3, 1200 h; TP6, d 3, 1900 h. Fecal pH was measured immediately after each collection with a portable pH meter (AP110 Fisher Scientific, Pittsburgh, PA), directly into the samples that were then stored at 20°C until analyzed. All samples were sent to a commercial laboratory for analysis. Fecal samples from TP1 to TP6 were analyzed for DM, CP, NDF, starch and ADL contents. Acid detergent lignin was used as a marker to calculate the apparent total-tract digestibility (Kotb and Luckey 1972) according to the following equations:

$$\text{X digestibility} = (1\text{-}([\text{ADL}]_{\text{diet}} \text{ x } [\text{X}]_{\text{faeces}})/([\text{ADL}]_{\text{faeces}} \text{ x } [\text{X}]_{\text{diet}})) \text{ x } 100\%$$

with, X digestibility = digestibility coefficient of specific nutrient X (i.e., NDF, starch or CP). The superscript indicated samples (i.e., diet or faeces samples) in which the nutrient or internal marker were quantified, as described above.

3.6. Behavioural Observations

To investigate behavioural outcomes in each trimester of gestation, 4 heifers from each group were randomly selected and housed in a single pen. Sub-sampling was done to reduce the labour intensity associated with human observation (Beauchemin, 2018). Animals were marked with different colours to distinguish groups, and the trained observer was positioned at least 15 m from the pen to ensure unobstructed views and avoid animal contact. Feeding behaviour and behavioural patterns were assessed by 24 h of direct live observation on days 70-71 (early trimester), 170-171 (mid trimester), and 270-271 (late trimester). Animals were observed every 60 seconds (Das and Das, 2007) during the following activities: Feeding, lying, rumination, and other activities (walking, drinking, or stereotypes).

During the last 10 days to calving, animals were observed at 3-min intervals over a 24-h period as described by DeVries et al. (2003). We defined rumination activity as the time animals engaged in cud chewing (either in a standing or lying position) without ingesting feed. The lying activity was defined as the period when the forelegs were fully lowered onto the carpal joints and the hindlegs, thigh, and abdomen were fully resting on the flat surface. Feeding time was the period spent by each animal at the feeding bunk. A total of 54,720 behavioural observations were recorded between early, mid, and late gestation.

3.7. Laboratory Analyses

Samples were characterized for chemical traits, as previously reported by Gallo et al. (2021). Ash was evaluated as a gravimetric residue following cremation at 550 °C for 2 hours (AOAC 1995, method 942.05), and ether extract (EE) was prepared using AOAC (1995) method 920.29. The DM was determined by gravimetric loss of free water by heating at 105 °C for 3 hours (1995). The Kjeldahl method (AOAC 1995, method 984.13) was used to determine the crude protein (CP, N x 6.25). According to Licitra et al. (1996) the soluble fraction of CP was calculated (stated on a DM basis).

Using the AnkomII Fiber Analyzer (AnkomTechnology Corporation, Fairport, NY, USA), the neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (ADL) were measured in accordance with the procedure outlined by VanSoest et al. (1991). A neutral detergent solution with sodium sulfite and a heat-stable amylase (activity of 17.400 Liquefon units/mL, Ankom Technology, Fairport, NY, USA) was used for the NDF analysis. For the residual ash content, the contents of NDF, ADF, and ADL were adjusted. Polarimetry (Polax 2L, Atago®, Tokyo, Japan) was used to measure starch. By placing nylon bags in the rumens of two cannulated dairy cows for 24 hours (24 h NDFD), the disappearance of NDF after that time was measured (Gallo et al., 2017). According to Weiss (1999), the NFC was calculated as follows:

$$\text{NFC (g/kg of DM)} = 100 - (\text{NDF} + \text{CP} + \text{ash} + \text{ether extract})$$

3.8. Statistical Analysis

Data analysis was performed using R statistical software (R Core Team, 2020). Descriptive statistics were calculated for all dependent variables, followed by a normality test using the Shapiro test in R. A multivariable mixed regression model was fitted to estimate associations between feeding, duration of feeding, and day of gestation (DG) within - and between - group treatments. Each dependent maternal variable studied (feed intake, biometric measurements, apparent total tract digestibility and behavioural patterns) was analysed as repeated measures within treatments using the following multivariable linear regression model:

$$Y_{ijkl} = \mu + FP_i + D_j + FP{*}D_k + ID_l + e_{ijkl}$$

where Y_{ijkl} was the independent variable; μ was the overall mean; FP was the fixed effect of gestation feeding program of pregnant heifers (2 levels, 0.6M and 1.8M); D was the fixed effect of gestation day of pregnant heifers; FR*D was the interaction between the effects of FP and D; ID was the random effect of individual heifers; e was the residual error. Differences in means were considered significant when the probability value of the ANOVA test was less than .05. Murtaugh (2014) stated that a probability value between .05 and .10 was seen as suggestive but unreliable. The Tukey's test for honestly significant differences was used to separate the least squares means when the predictor effects were deemed significant

3.9. RESULTS

3.9.1. DMI and maternal growth performance

Preliminary data analysis was performed on all maternal dependent variables by comparing response variables with or without the inclusion of pregnant heifers carrying male fetuses. The results obtained showed that no differences were observed for all dependent maternal variables when the model includes or excludes pregnant heifers carrying male fetuses. However, since the long-term goal of this study was to experiment on female progenies from dams used in this study, all heifers carrying male fetuses were excluded in all analysis.

The effects of nutrient restriction in the early trimester of pregnancy on maternal growth performance and DMI are presented in Tables 3.3a-c. By design, voluntary DMI, expressed in kilograms per day, was higher in control fed heifers than nutrient restricted fed heifers during the first 80 and 120 days of gestation (DG) ($P<0.01$). Nutrient restricted fed heifers in early gestation had lower ($P<0.01$) DMI at 140 and 265 DG but were similar to the control fed heifers at day 170 and 230 of gestation based on the DMI prediction models used. As expected, DMI in the first trimester of gestation was similar, and lower (4.34±0.01 and 4.38±0.01 kg/d for NR80 and NR120, respectively) in the nutrient-restricted fed heifers than the control (13.2±0.24 kg/d) fed heifers (Figure 3.2a).

Metabolizable energy (ME) intake during pregnancy was significantly dependent ($P<0.0001$) on the nutritional status of the heifers during early gestation (Figure 3.2b). Similar to DMI, control fed heifers had greater ME intake in early gestation than nutrient restricted fed heifers ($P<0.0001$). As expected, ME intake was higher in NR80 fed heifers than NR120 fed heifers at 120 d of gestation after being exposed to refeeding. However, from 170 d of gestation, ME intake was similar between the nutrient restricted fed heifers ($P>0.1$). At 230 d of gestation, all heifers showed similar ME intake ($P>0.1$). After exposure to refeeding, nutrient restricted fed heifers showed a decrease in ME intake at 230 and 250 d of gestation ($P<0.01$). Control fed heifers showed similar ME intake between 230 and 250 d of gestation ($P>0.1$), however, this surprisingly was not reduced as calving approached.

Between days 140 and 265 of gestation, DMI was similar for NR80 and NR120 groups. At day 170 of gestation, no difference in DMI was observed between control vs NR120 vs NR80, however, a higher DMI ($P=0.039$) was observed in control fed heifers than NR80 fed heifers. DMI was comparable between nutrient restricted fed heifers and control fed heifers at 230 d of gestation. However, control fed heifers had higher DMI than nutrient restricted fed heifers at 250 d of gestation ($P<0.01$). Autonomously of feeding program, there was no difference in DMI during the last 3 months of gestation ($P>0.1$).

Evaluation of DMI, expressed as a percentage of body weight, showed a linear difference at days 170, 230, and 265 of gestation between the control and nutrient restricted groups (Table 3.3a). DMI (%BW) decreased, especially in the third trimester of gestation ($P<0.01$) and was lower and similar in all groups at day 265 of gestation. When DMI (%BW) was evaluated at day 230, we observed a difference ($P<0.01$) between the control group and NR80 group, but no difference between the nutrient-restricted groups vs the control and NR120 vs control. Overall, we found that as gestation progressed and ~15 days to the start of calving, DMI (%BW) significantly decreased by 42, 71, and -13% in NR120, NR80, and the control, respectively.

The body size and growth rate of pregnant heifers are shown in Figure 3.3. The effects of nutrient-restricted feeding in early pregnancy on body weight of pregnant heifers were clearly significantly dependent on the day of gestation ($P<0.01$) and, to some extent, on the nutritional status of the animals during the early trimester of gestation. There was no difference in initial body weight between all groups or in final body weight between the nutrient-restricted groups. However, final body weight between the control and nutrient-restricted groups was significantly different (P=0.042). Over the entire gestation period, heifers in NR120, NR80, and the control gained 32, 26, and 42% of their body weight, respectively.

Comparable to body weight, an interaction effect (P<0.0001) between FP and DG was observed for BCS during pregnancy (Figure 3.4). BCS recorded before artificial insemination (Period 1) was similar among nutritional groups (P>0.1).

Between periods 2 and 4, which represents the day of insemination and the second pregnancy confirmation at 55 d of gestation, control fed heifers had higher BCS than their counterparts (P<0.0001). However, BCS at calving and post calving were similar among all heifers (P>0.1). Independent of the feeding program, BCS significantly reduced after calving (P<0.0001). Whereas BCS in control fed heifers was significantly higher (P<0.05) in periods before insemination, at insemination and at pregnancy confirmation at 55 d of gestation compared to BCS at calving, nutrient restricted heifers gained higher BCS (P<0.001) at calving (Figure 3.5).

Similar to the voluntary DMI, body weight was higher in the control fed heifers than the nutrient-restricted fed heifers during the first 120 days of gestation (P<0.01), and this persisted as gestation progressed. However, at d 230, body weight was similar between the control and NR120 fed heifers (P=0.122). Nonetheless, irrespective of the feeding program, at d 230 and 265 of gestation, body weights of heifers increased linearly until calving.

Although an interaction effect (P<0.01) between DG and our feeding program for ADG was detected, ADG evaluated on a specific DG did not differ significantly among treatments (P>0.1). When evaluating ADG independently of feeding program and DG, an observed indication of the effects of the feeding program (P=0.06) on daily gains at 140 and 170 d of gestation in the nutrient-restricted groups was detected, and this can be explained by the increased DMI shortly after a restricted feeding period.

As expected, heifers in the control group had greater (P<0.01) hip height, expressed in centimetres (cm), than heifers in the nutrient-restricted groups (Table 3.3b). Additionally, we observed an increase (P<0.001) in hip height as pregnancy progressed and an interaction effect between DG and the feeding program (P<0.001) was detected. Additionally, a positive association was found between hip height (cm) and body weight (r=0.79, P=0.001). Likewise, when assessing hip height in meters (m), an interaction between DG and the feeding program was detected (P=0.019).

Results of the present study showed a tendency for interaction (P=0.110) between DG and the feeding program on thoracic circumference (Table 3.3b). We speculated that thoracic circumference would increase linearly as gestation progressed regardless of the feeding program, and this is supported by the high positive correlation coefficient (r=0.81, P=0.001) between body weight and thoracic circumference.

No difference was observed in feed efficiency between FP x DG (P=0.578). However, when DG was considered separately, an improvement (P<0.001) in feed efficiency was evident between early -and- mid gestation (Table 3.3c).

Considering the gestation length, no statistical difference was found between the nutrient restricted groups and control. (Table 3.3c).

3.9.2. Apparent total-tract digestibility and fecal pH

Dry matter apparent total-tract digestibility had a significant interaction effect (P<0.001) between FP and DG (Table 3.4). Considering the window (d 70 of gestation) of our feeding program, nutrient restricted fed heifers had similar (P>0.1) DM apparent total-tract digestibility to the Control-fed heifers. However, at 120 d of gestation, NR80 fed heifers showed greater DM apparent total tract digestibility than Control fed heifers. However, at 70, 170, 230 and 250 d of gestation, DM apparent total-tract digestibility was similar (P>0.1) between nutrient restricted- and overfed - heifers. We observed the highest (P<0.01) value of DM apparent total-tract digestibility in NR- and Control-fed heifers at 120 and 200 d of gestation.

An interaction effect (P = 0.0006) was observed for CP apparent total-tract digestibility between FP and DG. Both nutrient restricted and Control-fed heifers showed the greatest (P<0.001) value in CP apparent total-tract digestibility at 120 and 200 d (Table 3.4). However, no difference (P>0.1) for CP apparent total-tract digestibility was observed at 70, 170, 230, or 250 d of gestation. No difference (P=0.23) was observed for starch apparent total-tract digestibility interaction between FR and DG (Table 3.4). However, we observed differences (P<0.01) between FP: the greatest (P<0.01) value of starch apparent total-tract digestibility was detected in NR-fed heifers. NDF total tract apparent digestibility had no significant interaction effect (P=0.134) among the nutritional groups. Unlike in DM digestibility, NDF total tract apparent digestibility was similar (P>0.1) at 70 d of gestation between nutrient restricted fed heifers and Control-fed heifers. In addition, the greatest (P<0.01) NDF apparent total-tract digestibility value was observed in heifers at 170 and 200 d of gestation, and while remaining similar (P>0.05) at 70, 120, 230 and 250 d of gestation. Fecal pH in control fed heifers was higher than NR80 fed heifers (P=0.042), however, similar when compared to NR120 fed heifers throughout gestation (P=0.861). An interaction was observed between NR120 and NR80 fed heifers (P=0.063). An interaction tendency (P=0.13) was observed for fecal pH (Figure 3.4a). Nonetheless, Fecal pH was highest (P<0.001) in the hours after morning feeding (Figure 3.4b).

3.9.3. Voluntary behavioural outcomes

The descriptive data of the voluntary behaviour in each trimester of pregnancy are shown in Table 3.5. Although this was a small sample, there were significant differences between experimental groups and DG (P<0.01) in rumination, feeding, lying down, and other activities of gravid heifers. Because DMI was limited in the nutrient-restricted groups in early gestation, less time was spent eating (<1 h/day). This meant less time ruminating and more time resting (P<0.01) compared to the control group. The nutrient-restricted heifers compensated for their low eating and rumination time by spending more time (~ 9.6 h/day) carrying out other activities.

Rumination time was much higher in gravid heifers receiving nutrient restriction during the first 80 days of gestation at 170 and 270 DG compared to the control and NR120 fed heifers (P<0.001). In contrast, feeding time improved but was significantly lower in NR80 gravid heifers as pregnancy progressed (P<0.01) than in the control and NR120 fed heifers. As expected, more time was spent lying as heifers approached calving, although a borderline significance (P=0.07) in lying time was observed for the nutrient-restricted fed heifers.

Time spent feeding and rumination, which would tend to decrease in late gestation, was not observed in this study. In fact, higher recumbency time was observed in the nutrient-restricted groups at the beginning of gestation, but it decreased significantly in the subsequent trimesters of gestation (P<0.01). The hourly variation of behavioural activities (Figure 3.5a-c) presented the traditional behavioural patterns of ruminants before and after each meal. We found an interaction between our experimental groups (P<0.01), DG and time of day for all behavioural observations.

3.9.4. Calves body measurements

The body weight of calves at birth were significantly different across experimental groups (P=0.017). Calves from control fed heifers were heavier than those from NR80 (Figure 3.6). However, we found a tendency for body weight at birth to differ between control calves and those from NR120 fed heifers (P=0.107). However, as calves aged, this difference disappeared (Figure 3.6). Similarly, heart girth (Figure 3.7a) was larger in the calves from control fed heifers than calves born to early gestation nutrient restricted heifers (P=0.049). A tendency to differ for hip height (Figure 3.7b) between calves from control fed heifers and nutrient restricted fed heifers was observed (P=0.069). However, wither height at birth (Figure 3.7c) was similar among all calves (P=0.677).

In Figures 3.8a-d, calves body measurements within the first 1 month of age are presented. Calves born to heifers used in this study had no interactive effect (P>0.1) between treatment (maternal feeding program) and age post-natal, in hip height, back length, and wither height. We observed no interaction effect in body weight and age after birth (P=0.313). Furthermore, no interaction effect (P=0.925) in average daily gain (ADG) was observed between the experimental groups and calves age (Figure 3.9).

3.10. DISCUSSION

The main findings of this study were: 1), maternal growth performance, dry matter intake and behavioural outcomes depend on the maternal nutritional status of gravid heifers in the first trimester of pregnancy. 2), Heifers exposed to early-gestation nutrient restriction compensated for losses in the following trimester of gestation regardless of the duration of restriction. 3), Calves born to heifers subjected to our feeding program [at either 1.8 or 0.6 of their energy maintenance requirements (M)] and duration of restriction influenced calf birth weight as control fed heifers had heavier calves than NR80 fed heifers. Hip height and heart girth also differed across nutritional groups but remained similar 30 d postnatal. 4), Maternal BCS was similar at calving irrespective of the feeding program, however, was significantly lower after parturition for all heifers. 5), Higher crude protein, dry matter and NDF nutrient apparent total tract digestibility was evident for heifers restricted to 0.6 of their maintenance energy requirements during the first 80 days of gestation than their counterparts at fed through the first 120 d of gestation, however, this ascendancy disappeared as pregnancy progressed. 6), all dependent maternal variables showed an interactive effect between the two predictors (feeding program and gestation day) in the model, suggesting that gestation day and feeding program had as much influence as the other in affecting all maternal response variables.

Based on literature sourced and current knowledge, this study is the first to characterize maternal behaviour, apparent total tract digestibility, growth patterns, and DMI in subsequent trimesters of pregnancy after exposure to different levels of maintenance energy requirements in the early trimester of pregnancy. In the present study, we expected that feeding heifers 0.6 of their maintenance energy requirements (M) would result in severe BW reduction, however, BW remained constant throughout the differential feeding period. This could be linked to compensatory growth through altered growth hormones (Lammers et al., 1999) or the greater nutrient digestibility coefficients observed in nutrient restricted heifers compared to the control. Generally, restricted feeding is associated with faster passage rate and increased mean retention time of digesta (Pino and Heinrichs, 2018) and feed efficiency (Erickson et al., 2020). According to research by Park et al. (1987) heifers who were fed less during compensatory growth had quicker body gains and improved protein and energy utilisation. As a result, it is likely that the constant growth rate observed in nutrient-restricted heifers during the differential feeding was caused by an increased protein digestibility, which in turn promoted protein turnover (Silva et al., 2017).

It is generally reported that DMI in adult cows decreases as pregnancy progresses (Rotta et al., 2015) and a greater decrease in DMI is well pronounced in the last 3-4 days prepartum (Pérez-Báez, et al., 2019). However, in growing gravid heifers, there is a lack of knowledge about the changes in DMI in the last trimester of pregnancy (NRC, 2001). When evaluating DMI (kg/d), we observed an increased DMI when heifers in the nutrient-restricted groups were exposed to re-alimentation from mid - to late – gestation (Table 3.3a). The reason for this may be that gravid heifers must provide nutrients to the growing fetus and rely on nutrients for their own growth and skeletal development. However, our results contrast the physiological response of mature or dried cows, that showed a 22% decrease in DMI (kg/d) in the last trimesters of gestation (Rotta et al., 2015). However, Tedeschi and Fox (2020) reported that when diet energy is the main limiting factor, rumen fill limits the intake which is consistent with the low DMI observed during the differential feeding phase between the nutrient restricted groups.

It is important to note that the TMR administered was adequate in terms of nutrient composition (NRC, 2001), and larger in particle size than is often recommended (peNDF of 21: Kmicikewycz et al., 2015). This level of dietary particle size minimized the risk of cows sorting out (Fustini et al., 2016) and suggests that any variation in intake after gravid heifers were exposed to refeeding may be physiological in nature and not due to diet.

In this study, we speculated that DMI expressed as a % of body weight, especially for the control group, would follow patterns like those reported in adult cows as heifers in the nutrient-restricted groups would continue to eat to restore the body condition needed to calve (Hoffman, 2017). However, heifers in all FP maintained a constant DMI from day 230 to 265 of gestation. The results obtained in this study on DMI are comparable to those reported by Periera et al. (2020) for Holstein heifers. When considering DMI (as a % of body weight), we observed a decrease in DMI with advancing pregnancy in all experimental groups, which is consistent with the results obtained in Holstein x Gry heifers (Rotta et al., 2015) and Holstein heifers (Pereira et al., 2020).

It is unclear why DMI (as % of body weight) decreased in the nutrient-restricted groups during the last trimester of gestation, considering that these heifers must metabolize nutrients for growth and fetal development. One theory could be that the fetal weight of about 58%, which occupies more than 70% of the stomach in the last trimesters of gestation (Ferrel et al., 1978b), may be responsible. This result suggests that fetal skeletal growth and development may not have been affected by our feeding program and could be linked to the compensatory growth (Park et al., 1987; Wathes, 2022) evidenced between mid to late gestation. Other studies have supported the reduction in physical rumen space by the fetus as a potential factor

(Hummel et al., 2021), which has indeed been reported as the major limiting factor affecting DMI in gravid ruminants (Forbes, 2009).

According to Clark et al. (2007), the level of intake influences apparent total-tract DM digestibility in beef steers, and limit feeding has been shown to increase digestibility of the diet compared with ad libitum feeding (Murphy et al., 1994; Clark et al., 2007; Rotta et al., 2015). However, in the present study, we observed that differences between the three FP occurred only from 70 to 120 d of gestation (Table 3.5).

Independently of the feeding program that was studied, we observed that apparent total-tract DM digestibility decreased with advancing gestation. Beharka et al. (1988), Rotta et al. (2015), and Pereira et al. (2020) also observed decreases in apparent total-tract DM digestibility in ewes, dairy cows, and dairy heifers, respectively. Beharka et al. (1988) observed that apparent total-tract DM digestibility decreased while the rate of passage increased, as expected. According to those authors, because the rate of passage and digestibility are competing forces, the decreased apparent total-tract DM digestibility may be explained in part by the increased passage rate. Linton and Allen (2007) suggested that the passage rate of NDF increased with increasing DMI. Heifers fed *ad libitum* are therefore supposed to have a greater passage rate of NDF than nutrient restricted-fed heifers, which likely explains the reduction in total-tract apparent digestibility of NDF.

In the present study, it was difficult to invasively measure the pH of the upper or lower gastrointestinal tract during pregnancy. However, fecal pH has been widely used as a surrogate for hindgut pH previously reported in sheep and steer (Wheeler and Noller, 1977), and recently proposed for dairy cows (Palladino et al., 2022). The hourly mean fecal pH dynamics observed in this study is consistent and higher than those reported by Palladino et al. (2022) for dairy cows even though lower fecal pH values have been reported in starch fed cows (Luan et al, 2016). Although the description of fecal pH as slightly acidic (Dirksen, 1986) contradicts our findings and other comparative data, it is still important to understand the various factors, such as feeding management, that can lead to pathological entrainment of fecal pH and its correlation with intestinal physiology and gut microbiota may be responsible for the differences in fecal pH in ruminants.

Nutrient restriction in the first trimester of gestation affected heifer behavior, with heifers in both the NR80 and NR120 groups ruminating below the minimum threshold of > 390 min/day to ensure rumen functionality (Zebeli et al., 2009). Similarly, feeding time was reduced to less than 1 hour per day, which would normally be 3-5 hours (Greter et al., 2008) or up to 9 hours under pasture conditions (Hafez and Bouissou, 1975). Although Hoffman et al. (2007) reported a similar result, this pattern of behavior is not consistent in the literature.

Rumination, which is a secondary function of gut health, appeared to improve significantly in the subsequent trimesters of pregnancy, indicating a healthy gut (Radostits et al., 2007; Paudyal, 2021) or that the physical effective fiber of the diet (Yang and Beauchemin, 2007) was adequate, among other factors. Rumination and feeding time have been widely reported to decrease in days leading up to calving (Matamala et al., 2021), 6 hours before calving (Miedema et al., 2011) or even on the day of calving (Schirmann et al., 2013). In this study, a decrease in feeding time and an increase in rumination time were observed in all experimental groups. The reason for the increased rumination time is unclear, but we can speculate that the increased rumination time on day 270 of gestation was a possible characteristic of the heifers to continuously support the metabolism of nutrients for both the growing fetus and the dam itself. Future studies should be conducted to evaluate these observed changes, especially the increased rumination time in growing heifers in the last days before calving.

In summary, the natural compensatory behavior of heifers that are nutrient restricted by limited feeding includes increased lying time (Hoffman et al., 2007), prolonged standing that can lead to foot pathologies in dairy cows (Cook et al., 2004; Ramanoon et al., 2018). Such effects of nutrient restriction may indicate impaired welfare (Valizadeh et al., 2008). Except for foot pathologies which were not primarily investigated, we observed similar responses in this study.

In support of our secondary hypothesis, heifers in the nutrient-restricted groups calved at > 80% of their adult weight, suggesting that adequate skeletal development was achieved regardless of the degree of nutrient restriction. The increase in body weight and ADG was much greater in the nutrient-restricted heifers (especially those in the NR120 group) between the second and third trimesters of gestation than in the control group, indicating a compensatory growth after being exposed to refeeding. Even while gravid heifers in the NR80 group were exposed to *ad libitum* feeding early in gestation after a brief period of nutrient restriction, body weight gain was similar to those in the NR120 throughout pregnancy. The reason for this could be an increased plasma leptin concentration, evidenced by increased backfat thickness that often promotes fat deposition rather than muscle growth, and this may be the cause of 'compensatory weight gain (Ford et al., 2007), as shown in this current study.

Exclusive of the feeding program, body condition score in the current study decreased after calving. This is consistent with results obtained for dairy cows (Roche et al., 2009; Chebel et al., 2018). Reduced BCS at calving is linked to decreased milk production and a lesser chance of becoming pregnant, whereas elevated BCS at calving is linked to a higher risk of postpartum metabolic disorders (Roche et al., 2009). Interestingly, in the current study, heifers in the nutrient restricted group had elevated BCS during re-feeding and at calving. One explanation could be that once heifers recovered from negative energy balance after the period of restriction in early gestation, they gained elevated BCS leading up to calving. This condition has been previously reported for dairy cows during mid to late lactation after recovering from negative energy balance in early lactation (Mishra et al., 2016).

However, dairy cows with higher or lower BCS at calving typically have significant energy loss during the dry period, which causes reproductive, metabolic, and productive problems (Souissi and Bouraoui, 2019). Nevertheless, BCS at calving was within the normal range reported in literature indicative of normal growth rate and energy balance (Ohnstad, 2013).

In agreement with the results of the current study, Scheaffer et al. (2001) reported that increasing body weight and maintaining an ADG > 0.7 kg/d serve to support the growing fetus and meet nutrient requirements for mammary gland development. While it is questionable whether a maternal ADG of 1.0 kg/d in late gestation can be attributed to that of the fetus, although it has been reported that the fetus gains 0.5 kg daily during the last week of gestation (Muller et al., 1975), maternal body weight shortly after calving may be an effective way to evaluate this assumption.

Contrary to the hypothesis of the current study, the gestational length among all groups was similar. However, female calves from the control fed heifers were heavier at birth than NR80 calves and a tendency to differ from the NR120 fed group. Because birth weight has been associated with gestational length in heat-stressed cattle (Tao et al., 2012), we speculated that heifers with long (severe) exposure to maternal nutrient restriction in early gestation will trigger quicker calving than their counterparts. Considering this, our results contrast those of Tao et al. (2012), who found that the lower birth weight (~6 kg less) of calves from heat stressed cows may be due to shorter gestation length induced by environmental stress during the last 45 days to calving. However, multiple recent research (Laporta et al., 2017; Dado-Senn et al., 2020a; Dado-Senn et al., 2021) have shown that late gestation heat stressed cows give birth to lighter calves and that this can have long-term effects like reduced milk production in their first lactation cycle (Laporta et al., 2020).

Since alterations in maternal nutrition during gestation is a growing body of evidence explaining the concept ‘fetal programming’ (Vonnahme et al., 2003; Mossa et al., 2013), the difference for calves’ body weight between birth and 1 month post calving is consistent with results obtained in cattle when maternal energy requirement was altered in early (Mickle et al., 2010), or mid – late (Radunz et al., 2012) gestation in cattle.

Average daily gains of calves during the first 4 weeks of postnatal life were within the range reported in the literature for young calves (Bazeley et al., 2016), however, below the target gains of > 0.75 kg/d required to achieve desired future calf production performances and minimize economic loss (MacDonald et al., 2005) particularly for the progenies whose mothers were nutrient restricted. Because an important indicator of ADG in the preweaning phase links to a reasonable extent, the maternal BCS gained during gestation and how it can impact calves’ growth in the preweaning stage (Moriel et al., 2021), BCS in this study was significantly affected by the feeding program heifers were subjected to. In addition, calves ADG is one of the ways to measure the health status of these animals (Hyde et al., 2021) since a considerable percentage of calf mortality is recorded in the preweaning period (Hyde et al., 2022).

Biometric parameters such as ADG, hip height, and body condition scores, among others, during gestation could serve as useful predictors of calving and calf birth weight (NRC, 2000; Duplessis et al., 2015; Rodríguez-Sánchez et al., 2017). Additionally, calf body measurements and growth performances can be useful indicators of future dairy cow performance (Soberon et al., 2012; Shivley et al., 2018) and can be useful in making informed management decisions on both farm and animal level (Wilson et al., 1997). Therefore, adequate maternal skeletal development is required to avoid calving difficulties as well as improve fetal skeletal frame.

Despite the biological importance of maternal energy restrictions in early gestation (Diniz et al., 2021a) or the economic benefits of limit heifer feeding (NRC, 2001), defining accurate feeding strategies during gestation is still a complex task. Although no detrimental effects of nutrient restrictions were found in this study, we recommend further studies to investigate maternal performance during lactation and the next breeding windows, as well as long-term or even multigenerational effects on overall calf performance.

3.11. CONCLUSION

The findings from this study suggests that regardless of the duration of nutrient restriction to which dairy heifers were subjected in the first trimester of pregnancy, these animals compensated for nutrient losses to a large extent in subsequent trimesters of pregnancy without compromising body or skeletal development. Independently of the feeding program studied, DM, NDF and CP apparent total tract digestibility decreased with advancing gestation. However, compared to the control, nutrient restricted heifers had greater nutrient CP digestibility during the differential feeding period. Contrary to the hypothesis, the feeding program used in this study did not affect gestation length; however, control fed heifers produced heavier calves at birth. Furthermore, regardless of feeding program, heifers lost body condition score after parturition even though heifers in the nutrient restricted group had significantly lower BCS throughout gestation. This study was subject to limitations, including small sample size for behavioural observations and our inability to measure voluntary DMI in subsequent trimesters of pregnancy. Therefore, further research to examine maternal voluntary DMI in subsequent trimesters of pregnancy after periods of feed restriction and on behavioural outcomes with a larger sample is recommended.

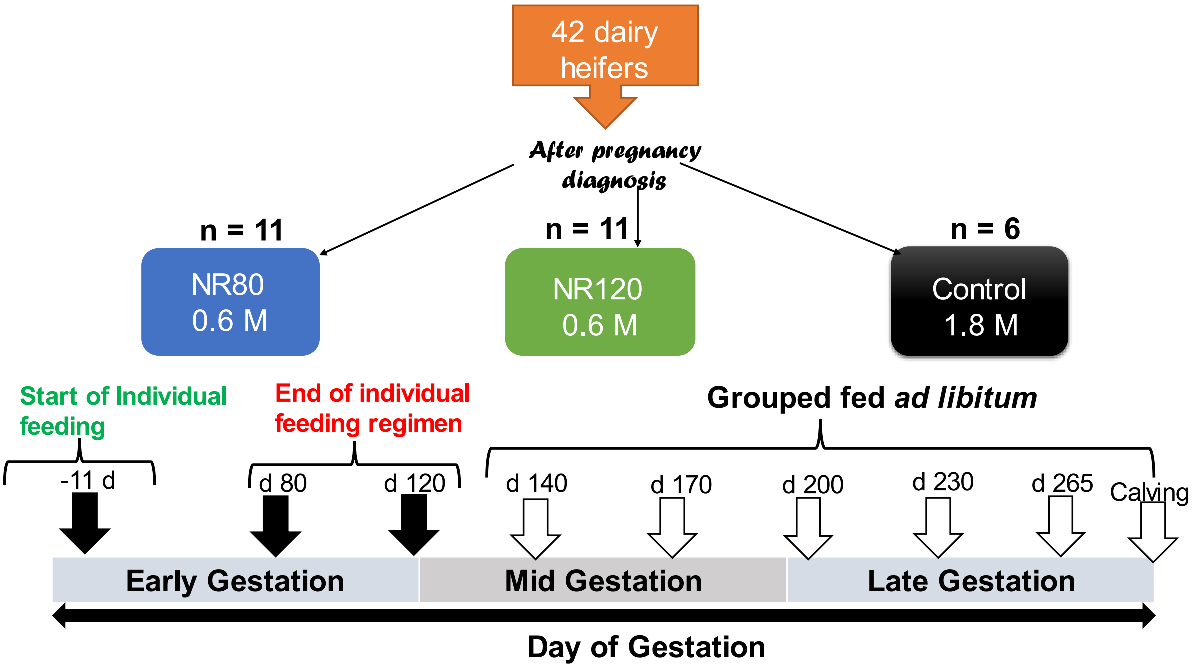


Figure 3.1. Schematic representation of the experimental protocol. Black arrows represent the commencement and end of an individual feeding program, while the white arrows indicate duration of grouped feeding and respective days for biometric measurements or behavioural observations. Control fed heifers received 1.8 of their maintenance energy requirements during the first 120 days of gestation, while nutrient restricted fed heifers (NR80 and NR120) received 0.6 of their maintenance energy during the first 80 and 120 days of gestation.

Table 3.1. Estrus synchronization, pregnancy confirmation and percentage, in the control and nutrient restricted groups.

Feeding groups	Synchronized heifers	Fertilized heifers	Pregnant heifers at 28 days	Pregnant heifers at 55 days
Total heifers	42	40	29 (72%)	28 (70%)
NR	32	31	23 (74%)	22 (71%)
Control	10	9	6 (66%)	6 (66%)

NR= Nutrient restricted

Table 3.2. Chemical composition of experimental diets (% DM)

Item	Dry TMR (Differential feeding)	Wet TMR (Post differential feeding)
DM	96.6	95.2
CP	14.5	10.9
NDF	45.3	48.7
ADF	30.2	32.6
EE	1.4	2.5
ADL	4.3	4.2
Ash	9.7	9.0
ADIP	1.0	0.9
Sugars	5.2	4.6
SOLP	4.2	4.9
NDIP	3.2	1.8
NDF24	48.8	48.3
Starch	16.3	11.6

Table 3.2. Continued

Item	Dry TMR (Differential feeding)	Wet TMR (Post differential feeding)
[1]NFC	29.1	28.9
[2]ME (Mcal/kg)	2.28	2.26
PSPS (Mean±SD*		
19 mm (%)	12.9±1.99	28.3±9.09
8 mm (%)	26.9±4.29	42.4±7.77
4 mm (%)	19.1±0.99	14.9±1.1
Bottom (%)	41.2±2.67	14.4±4.13
peNDF	**27.44**	**31.60**

[1] Calculated as NFC = 100 − CP − ash − NDF − ether extract.

[2] Calculated using NRC, 2001 guidelines.

*Penn State Particle Separator (PSPS). Dry TMR contained 34.5% grass hay, 19.2% steam flaked corn, 3.9% cane-beet molasses blend, and 42.4% grain mix {29.6% wheat bran, 29.4% sorghum grain, 21.6% soybean meal, 14.7% flaked soybean, 2.2% calcium carbonate, 1% sodium chloride, 0.4% magnesium oxide, 0.9% sodium bentonite, and 0.3% vitamin and mineral premix [provided 40,000 IU of vitamin A, 4,000 IU of vitamin D3, 30 mg of vitamin E 92% α-tocopherol, 5 mg of vitamin B1, 3 mg of vitamin B2, 1.5 mg of vitamin B6, 0.06 mg of vitamin B12, 5 mg of vitamin K, 5 mg of vitamin H1 (para-aminobenzoic acid), 150 mg of vitamin PP (niacin), 50 mg of choline chloride, 100 mg of Fe, 1 mg of Co, 5 mg of I, 120 mg of Mn, 10 mg of Cu, and 130 mg of Zn]}. Wet TMR (kg/DM) contained 4.01 kg ryegrass hay, 2.40 kg of ryegrass silage, 0.43 kg of ground fine corn, 0.88 kg Soybean meal 48%, 0.10 kg 4020 optimizer complex, 2.18 kg mineral premix. NDIP= neutral detergent insoluble protein, ADIP= acid detergent insoluble protein, SOLP= soluble protein

Table 3.3a. DMI in heifers exposed to early gestation maternal energy restriction (0.6M) vs Control (1.8M) in subsequent trimesters of pregnancy.

Item	Day of gestation (DG)											P-value		
	-59 (n=24)	12 (n=24)	71 (n=24)	81 (n=24)	101 (n=24)	120 (n=24)	140 (n=24)	170 (n=24)	200 (n=24)	230 (n=24)	265 (n=24)	FP	DG	FP x DG
DMI (kg/d)												***	***	***
Control	-	12.9 ±0.5^{a,AB}	12.7 ±0.4^{a,BC}	12.5 ±0.2^{a,BC}	13.5 ±0.4^{a,AB}	13.9 ±0.3^{aA}	10.8 ±0.1^{a,D}	11 ±0.1^{a,D}	11.1 ±0.1^{a,D}	11.1 ±0.2^{a,D}	11.8 ±0.3^{a,CD}			
NR80	-	4.4 ±0.1^{b,F}	4.4 ±0.1^{b,F}	8.7 ±0.1^{b,E}	8.9 ±0.1^{b,DE}	9.2 ±0.1^{b,CD}	9.6 ±0.1^{b,BC}	10 ±0.2^{b,AB}	10.1 ±0.1^{b,A}	10.3 ±0.1^{b,A}	9.6 ±0.3^{b,BC}			
NR120	-	4.4 ±0.1^{b,D}	4.4 ±0.1^{b,D}	4.4 ±0.1^{b,D}	4.4 ±0.1^{c,D}	4.4 ±0.1^{c,D}	9.7 ±0.1^{b,C}	10.1 ±0.1^{b,B}	10.3 ±0.1^{b,AB}	10.6 ±0.1$^{a.A}$	10.4 ±0.2^{b,AB}			
DMI (% BW)												***	***	***
Control	-	3 ±0.1^{a,A}	2.4 ±0.1^{a,A}	2.3 ±0.1^{a,B}	2.4 ±0.04^{aC}	2.4 ±0.04^{a,C}	1.8 ±0.03^{b,D}	1.7 ±0.03^{b,D}	1.7 ±0.03^{b,D}	1.7 ±0.04^{a,D}	1.7 ±0.02^{a,D}			
NR80	-	1.2 ±0.02^{b,B}	1.1 ±0.03^{b,G}	2.3 ±0.03^{b,G}	2.2 ±0.03^{b,A}	2.2 ±0.1^{b,AB}	2.1 ±0.03^{a,B}	2 ±0.04^{a,C}	1.9 ±0.04^{a,D}	1.8 ±0.04^{b,E}	1.6 ±0.03^{b,F}			
NR120	-	1.1 ±0.02^{b,B}	1.1 ±0.02^{b,E}	1.1 ±0.02^{b,E}	1.1 ±0.02^{b,E}	1 ±0.02^{b,F}	2.1 ±0.03^{a,A}	1.9 ±0.03^{a,B}	1.9 ±0.03^{a,B}	1.8 ±0.03^{ab,C}	1.7^{a,D}			

Table 3.3a Continued

	-59 (n=24)	12 (n=24)	71 (n=24)	81 (n=24)	101 (n=24)	120 (n=24)	140 (n=24)	170 (n=24)	200 (n=24)	230 (n=24)	265 (n=24)	FP	DG	FP x DG
DMI (g/kg)												***	***	***
Control	-	5.5 ±0.3^{a,E}	6.7 ±0.4^{a,E}	6.8 ±0.3^{a,CD}	7.5 ±0.4^{a,CD}	8.2 ±0.4^{a,AB}	6.7 ±0.3 ^{a,CD}	7.1 ±0.3^{a,CD}	7.3 ±0.3 ^{a,BCD}	7.3 ±0.4^{a,BCD}	8.3 ±0.4^{a,A}			
NR80	-	1.6 ±0.1^{b,G}	1.7 ±0.1^{b,G}	3.3 ±0.2^{b,F}	3.6 ±0.1^{b,EF}	3.9 ±0.1^{b,E}	4.5 ±0.2 ^{b,D}	5.1 ±0.3^{b,C}	5.3 ±0.2 ^{b,BC}	5.9 ±0.2^{b,A}	5.7 ±0.3 ^{b,AB}			
NR120	-	1.8 ±0.1 ^{b,D}	1.8 ±0.1^{b,D}	1.8 ±0.1^{b,D}	1.8 ±0.1^{b,D}	1.9 ±0.1^{b,D}	4.5 ±0.2	5.3 ±0.2^{b,B}	5.7 ±0.2^{b,B}	6.2 ±0.2^{b,A}	6.3 ±0.3^{b,A}			

***= P≤0.001, a–c Means within a column with different superscripts differ ($P \leq 0.05$). FP = feeding regimen; DG = day of gestation; FP × DG = interaction between feeding program and day of gestation. NR80: n=10, 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120: n=9, = 0.6 M until day 120 of gestation and Control: n=5. = ad libitum (1.8 M) until day 120 of gestation. $^{A\text{-}E}$Means within a row with different superscripts differ ($P \leq 0.001$).

Table 3.3b. Growth performance and stature in heifers exposed to early gestation maternal energy restriction (0.6M) vs Control (1.8M) at different day of gestation

	Day of gestation (DG)											P-value		
Item	-49 (n=24)	12 (n=24)	71 (n=24)	81 (n=24)	101 (n=24)	120 (n=24)	140 (n=24)	170 (n=24)	200 (n=24)	230 (n=24)	265 (n=24)	FP	DG	FP x DG
ADG (kg/d)												***	***	***
Control	-	0.7 ±0.2^{a,B}	1.4 ±0.2^{a,A}	1 ±0.2^{a,AB}	0.7 ±0.1^{a,B}	1.5 ±0.2^{a,A}	1.3 ±0.1^{b,AB}	1 ±0.2^{b,AB}	0.6 ±0.2^{b,B}	0.7 ±0.3^{b,B}	1.3 ±0.5^{a,AB}			
NR80	-	0.1 ±0.2^{b,CD}	0.1 ±0.2^{b,CD}	-0.2 ±0.2^{b,D}	0.9 ±0.2^{b,ABCD}	1.1 ±0.1^{b,ABC}	1.9±0.2^{a,A}	1.5 ±0.3^{a,AB}	0.6 ±0.1^{b,BCD}	1 ±0.4^{a,ABC}	0.8 ±0.4^{b,ABCD}			
NR120	-	0.3 ±0.3^{b,CDE}	-0.1 ±0.3^{b,DE}	-0.4 ±0.2^{b,E}	0.6 ±0.2^{b,BCDE}	1.2 ±0.1^{b,ABC}	1.4 ±0.1^{a,AB}	1.7 ±0.2^{a,A}	1.1 ±0.3^{a,ABC}	1 ±0.2^{ab,ABCD}	0.7 ±0.2^{b,ABCD}			
Hip height (cm)												***	***	***
Control	-	-	148.5 ±1.5^{a,E}	148.5 ±1.5^{a,E}	149 ±1.6^{a,DE}	149.3 ±1.5^{a,DE}	150.7 ±1.3^{a,CD}	151.3 ±1.5^{a,BC}	152 ±1.5^{a,ABC}	152.7 ±1.3^{a,AB}	153.7 ±1.3^{a,A}			
NR80	-	-	140.3 ±0.9^{b,E}	140.3 ±0.9^{b,E}	141.1 ±1.2^{b,DE}	141.8 ±1.2^{b,DE}	140.8 ±1.1^{b,DE}	142.8 ±1^{b,CD}	144.7 ±1.2^{b,BC}	146.2 ±1.1^{b,AB}	147.8 ±1.3^{b,A}			
NR120	-	-	139.5 ±0.5^{b,D}	139.5 ±0.5^{b,D}	140.4 ±0.8^{b,D}	141.7 ±0.1^{b,CD}	143.6 ±1^{b,BC}	144.5 ±0.9^{b,B}	145.6 ±1.1^{b,B}	148.4 ±1^{ab,A}	148.4 ±1.4^{b,A}			

Table 3.3b Continued

Hip height (m)												0.158	0.019	0.019
Control	-	-	1.5 ±0.02 ^{a,A}	1.5 ±0.01 ^{a,A}	1.5 ±0.02 ^{a,A}	1.5 ±0.02 ^{a,A}	1.5 ±0.01 ^{a,A}	1.5 ±0.02 ^{a,A}	1.5 ±0.02 ^{a,A}	1.5 ±0.01^{a,A}	1.5 ±0.01^{a,A}			
NR80	-	-	1.4 ±0.01^{a,E}	1.4 ±0.01^{a,E}	1.4 ±0.01 ^{a,DE}	1.4 ±0.01 ^{a,DE}	1.4 ±0.01 ^{a,DE}	1.4 ±0.01 ^{a,CD}	1.4±0.01 ^{a,BC}	1.5 ±0.01 ^{a,AB}	1.5 ±0.01^{a,A}			
NR120	-	-	1.4 ±0.01 ^{a,D}	1.4 ±0.01 ^{a,D}	1.4 ±0.01 ^{a,D}	1.4 ±0.01 ^{a,CD}	1.4 ±0.01 ^{a,BC}	1.4 ±0.01 ^{a,B}	1.4±0.01 ^{a,B}	1.5 ±0.01^{a,A}	1.5 ±0.01^{a,A}			
TC (cm)												***	***	0.111
Control	-	173.6 ±1.1^{a,F}	187.3 ±1.8^{a,E}	186.9 ±1.9^{a,E}	188.8 ±1.6^{a,DE}	193.4 ±1.9 ^{a,CD}	195.8 ±1.9 ^{a,BC}	199.5 ±1.8 ^{a,AB}	201 ±1.8 ^{a,A}	201.2 ±2.6^{a,A}	203.9 ±2.1 ^{a,A}			
NR80	-	166.2 ±1.5^{a,EF}	167.7 ±1.6^{b,EF}	167.6 ±1.6 ^{a,EF}	170.8 ±1.1^{a,DE}	173.9 ±1.6 ^{a,D}	179.8 ±1.6 ^{a,C}	185.8 ±2.4 ^{a,B}	185.2 ±1.9 ^{a,AB}	188.7 ±1.8^{a,AB}	190.1 ±1.8 ^{a,A}			
NR120	-	169.4 ±1.7 ^{a,BCD}	170.4 ±1.6 ^{a,BCD}	154.5 ±15.4 ^{a,D}	174.5 ±2.2 ^{a,ABCD}	177.2 ±1.9 ^{a,ABC}	182.9 ±1.6 ^{a,ABC}	185.7 ±1.4 ^{a,ABC}	188.7 ±1.6 ^{a,AB}	192.8 ±1.6^{a,A}	195.1 ±1.5 ^{a,A}			

***= $P \leq 0.001$, a–c Means within a column with different superscripts differ ($P \leq 0.05$). FP = feeding regimen; DG = day of gestation; FP × DG = interaction between feeding program and day of gestation, TC = Thoracic circumference. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. A-F Means within a row with different superscripts differ ($P \leq 0.001$)

Table 3.3c. Heifers gestation length and feed conversion ratio from early gestation maternal energy restriction (0.6M) vs Control (1.8M)

Item	Day of gestation (DG)											P-value		
	-59 (n=24)	12 (n=24)	71 (n=24)	81 (n=24)	101 (n=24)	120 (n=24)	140 (n=24)	170 (n=24)	200 (n=24)	230 (n=24)	265 (n=24)	FP	DG	FP x DG
Gestation length (days)												NS	-	-
Control											277 ±1.6			
NR80											274 ±1.2			
NR120											274 ±0.9			
F:C												0.07	***	0.735
Control	-	0.1 $\pm0.2^{a,A}$	0.4 $\pm0.1^{a,A}$	1.6 $\pm0.5^{a,A}$	1.1 $\pm0.3^{a,A}$	0.5 $\pm0.1^{a,A}$	0.4 $\pm0.1^{a,A}$	0.4 $\pm0.1^{a,A}$	2.5 $\pm1.7^{a,A}$	1.3 $\pm0.4^{a,A}$	2.2 $\pm1.8^{a,A}$			
NR80	-	-0.3 $\pm0.5^{a,AB}$	-0.7 $\pm0.5^{a,AB}$	-1.5 $\pm1^{a,B}$	-1 $\pm1^{a,AB}$	0.5 $\pm0.1^{a,AB}$	0.3 $\pm0.1^{a.AB}$	0.4 $\pm0.2^{a,AB}$	2.1 $\pm1.1^{a,A}$	1.7 $\pm1.1^{a,AB}$	1.3 $\pm1^{a,AB}$			
NR120	-	0.2 $\pm0.1^{a,A}$	0.2 $\pm0.5^{a,A}$	-0.5 $\pm0.5^{a,A}$	0.3 $\pm0.2^{a,A}$	0.2 $\pm0.01^{a,A}$	0.4 $\pm0.1^{a,A}$	0.2 $\pm0.4^{a,A}$	2.2 $\pm1.3^{a,A}$	1.2 $\pm0.1^{a,A}$	2.1 $\pm1.3^{a,A}$			

***= $P\leq0.001$, a–c Means within a column with different superscripts differ ($P \leq 0.05$). A-B Means within a row with different superscripts differ ($P \leq 0.001$). FP = feeding regimen; DG = day of gestation; FP × DG = interaction between feeding program and day of gestation, F:C = Feed conversion, NS: not significant. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

Table 3.4. Apparent total tract digestibility (%) in heifers fed 1.8M or 0.6 M of their maintenance energy requirement in early gestation

Item	Feeding program (FP)			Day of gestation (DG)						FP	DG	FP x DG
	Control (n=5)	NR80 (n=9)	NR120 (n=10)	70 (n=24)	120 (n=24)	170 (n=22)	200 (n=23)	230 (n=21)	250 (n=20)			
DM	80.8±0.4^{b}	82.2±0.4^{a}	81.5±0.3^{b}							<0.0001	<0.0001	<0.0001
Control				78.3± 0.6^{a,C}	83± 0.4^{b,AB}	81.9± 0.3^{a,AB}	83.6± 0.4^{a,A}	80± 0.4^{a,BC}	79.4± 0.9^{a,C}			
NR120				79.7± 0.3^{a,C}	84± 0.3^{b,AB}	81.8± 0.1^{a,B}	84± 0.2^{a,A}	80± 0.4a,C	80± 0.5^{a,C}			
NR80				79.9± 0.4^{a,D}	86.2± 0.2^{a,A}	81.9± 0.3^{a,C}	83.7± 0.2^{a,B}	82.1± 0.3^{a,BC}	79.9± 0.6^{a,D}			
CP	83.5±0.7^{b}	85±0.6^{a}	84.4±0.5^{ab}							0.00009	<0.0001	0.0006
Control				81.7± 0.3^{b,CD}	89.4± 0.2^{b,A}	80.3± 0.9^{a,B}	86.8± 0.2^{a,C}	83.1± 1.1^{a,D}	80.4± 0.5^{a,D}			
NR120				84.3± 0.2^{a,C}	91± 0.1^{b,A}	80.1± 0.4^{a,E}	86.6± 0.1^{a,B}	82.7± 0.7^{a,CD}	81.3± 0.6^{a,DE}			
NR80				84.5± 0.3^{a,C}	92.3± 0.1^{a,A}	79.8± 0.4^{a,D}	87± 0.2^{a,B}	85± 0.2^{a,C}	81.2± 0.6^{a,D}			

Table 3.4. Continued

Item	Feeding program (FP)			Day of gestation (DG)						FP	DG	FPxDG
	Control (n=5)	NR80 (n=9)	NR120 (n=10)	70 (n=24)	120 (n=24)	170 (n=22)	200 (n=23)	230 (n=21)	250 (n=20)			
NDF	72.4±0.4^{b}	73.4±0.2^{a}	72.9±0.2^{ab}							0.008	<0.0001	0.085
Control				74.4±1.1^{a,B}	71.7±0.6^{a,AB}	74.1±0.3^{a,A}	74.4±0.4^{a,A}	71.3±0.3^{a,AB}	72.4±1.3^{a,AB}			
NR120				72.2±0-3^{a,BC}	72.3±0.6^{a,BC}	73.5±0.2^{a,B}	75.1±0.2^{a,A}	71.6±0.2^{a,C}	72.5±0.6^{a,BC}			
NR80				72.3±0.4^{a,B}	74.2±0.4^{a,AB}	74.1±0.4^{a,AB}	74.5±0.3^{a,A}	73.2±0.7^{a,AB}	72.4±0.7^{a,B}			
Starch	98.1±0.1^{b}	98.5±0.1^{a}	98.5±0.1^{a}							0.006	<0.0001	0.230
Control				98.1±0.2^{a,B}	98.9±0.1^{a,A}	98.2±0.2^{a,AB}	98.8±0.03^{a,A}	97.9±0.4^{a,BC}	97.1±0.1^{a,C}			
NR120				98.1±0.1^{a,C}	99±0.04^{a,A}	98.6±0.1^{a,AB}	98.7±0.1^{a,AB}	98.3±0.2^{a,BC}	97.9±0.2^{a,C}			
NR80				98.2±0.1^{a,C}	99.3±0.03^{a,A}	98.5±0.1^{a,BC}	98.8±0.05^{a,B}	98.5±0.2^{a,BC}	97.7±0.2^{a,D}			

^{a,b}Means within a column with different superscripts differ ($P \leq 0.01$). $^{A\text{-}D}$Means within a row with different superscripts differ ($P \leq 0.01$).

FP= Feeding program; DG = day of gestation; FR × DG = interaction between feeding program and day of gestation. CP = Crude protein, DM = Dry matter, NDF = Neutral detergent fibre. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean.

Table 3.5. 24 hours behavioural outcomes of gravid heifers fed either 1.8M or 0.6 M of their maintenance energy requirement in early gestation

Item (Mins/day)	Feeding program (FP)			Day of gestation (DG)			P-value		
	Control (n=5)	NR80 (n=4)	NR120 (n=4)	65	170	270	FP	DG	FP x DG
Feeding	297^{a}	229^{c}	248^{b}				0.002	***	***
Control				$302^{a,B}$	$245^{b,C}$	$343^{a,A}$			
NR80				$49^{b,C}$	$343^{a,A}$	$295^{b,B}$			
NR120				$51^{b,B}$	$351^{a,A}$	$343^{a,A}$			
Rumination	503^{a}	484^{a}	476^{a}				NS	***	***
Control				$471^{a,B}$	$459^{b,B}$	$578^{b,A}$			
NR80				$288^{b,C}$	$506^{a,B}$	$659^{a,A}$			
NR120				$274^{b,C}$	$540^{a,B}$	$614^{b,A}$			
Lying	434^{a}	414^{b}	410^{b}				0.03	***	***
Control				$400^{b,C}$	$553^{a,A}$	$350^{a,C}$			
NR80				$529^{a,A}$	$362^{a,B}$	$352^{a,B}$			
NR120				$536^{a,A}$	$377^{a,B}$	$317^{a,C}$			
Others	206^{b}	312^{a}	307^{b}				0.001	***	***
Control				$264^{b,A}$	$184^{b,B}$	$171^{a,B}$			
NR80				$574^{a,A}$	$229^{a,B}$	$134^{b,C}$			
NR120				$581^{a,A}$	$173^{b,B}$	$166^{a,B}$			

***= $p \leq 0.05$, a–c Means within a column with different superscripts differ ($P \leq 0.05$). A–C Means within a row with different superscripts differ ($P \leq 0.05$). FP = feeding regimen; DG = day of gestation; FP × DG = interaction between feeding program and day of gestation. Others = all other activities. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

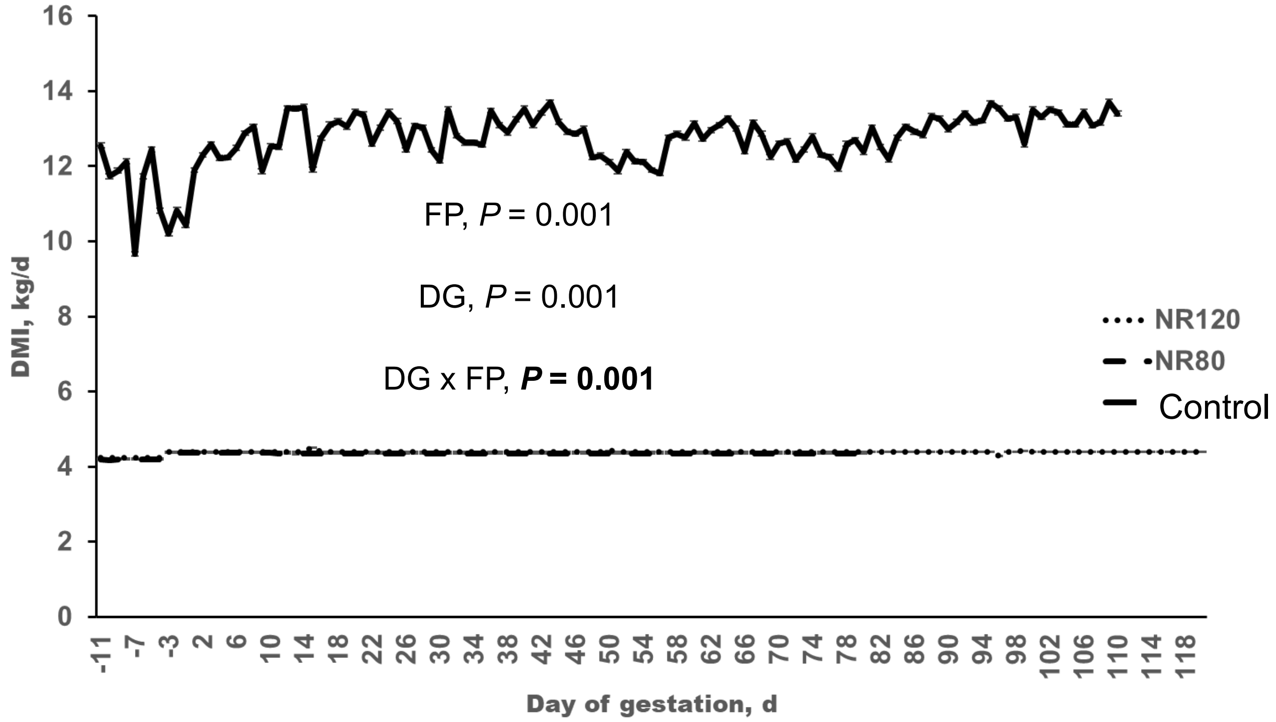


Figure 3.2a. DMI expressed in kilograms per day of pregnant heifers (n=24) in the first trimester of pregnancy. FP = Feeding program, DG = Day of gestation. Control; Fed 1.8M of their maintenance energy requirement during the first 120 d of gestation, NR80 = Fed 0.6M of their maintenance energy requirement during the first 80 d of gestation, NR120 = Fed 0.6M of their maintenance energy requirement during the first 120 d of gestation.

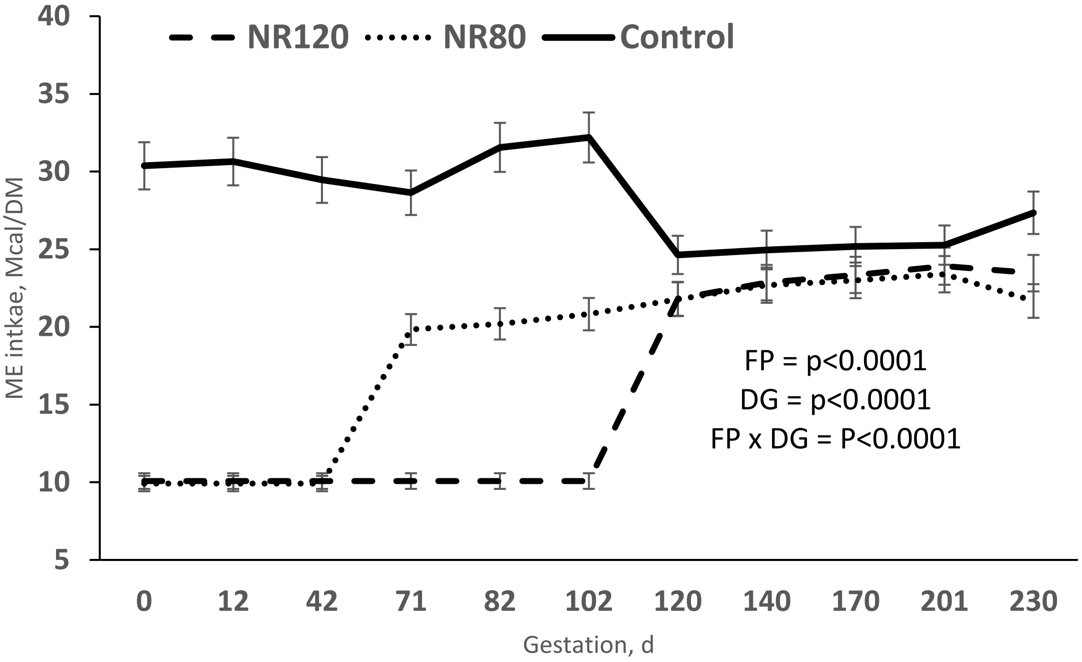


Figure 3.2b. Metabolizable energy intake in pregnant heifers (n=24). Error bars represents standard error of the mean. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

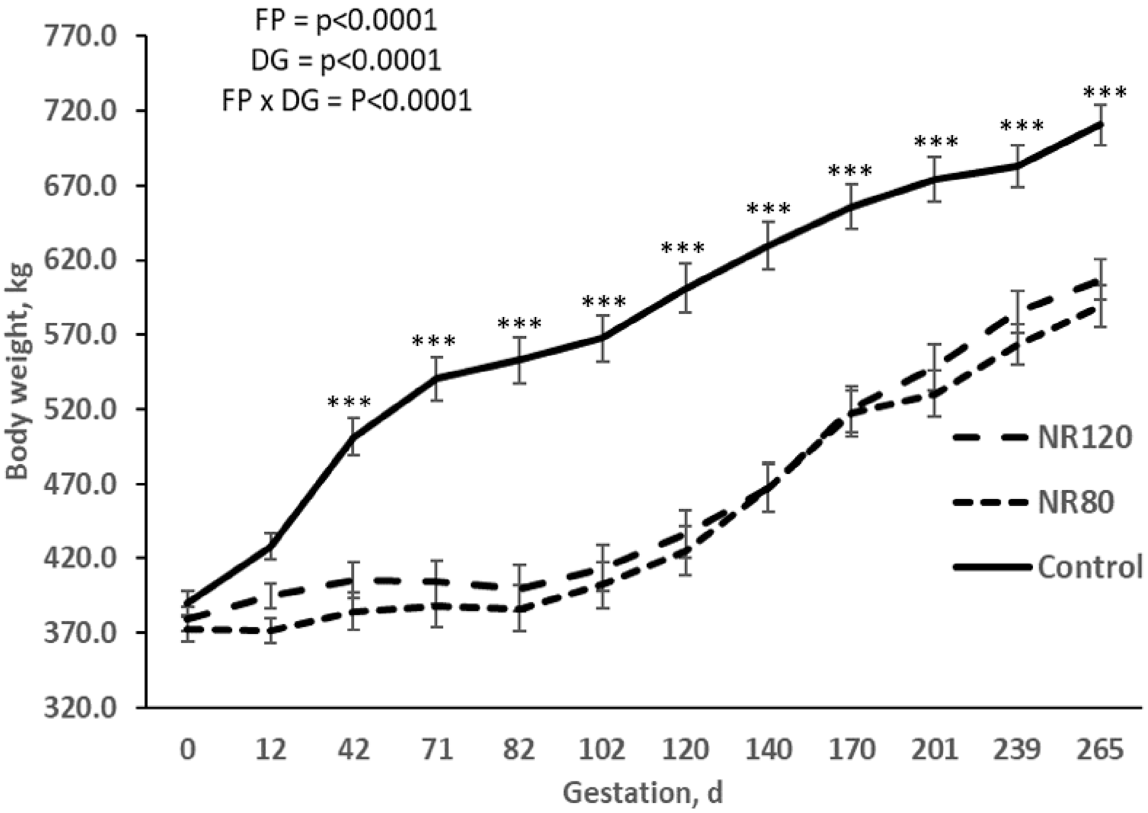


Figure 3.3. Body weight of pregnant heifers (n=24) from day -59 to day 265. -59 d was considered day 0. The effects of the feeding program (FP) [1.8M vs O.6M], day of gestation and the interaction were significant (p<0.0001). FP = Feeding program, DG = Day of gestation. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of the mean.

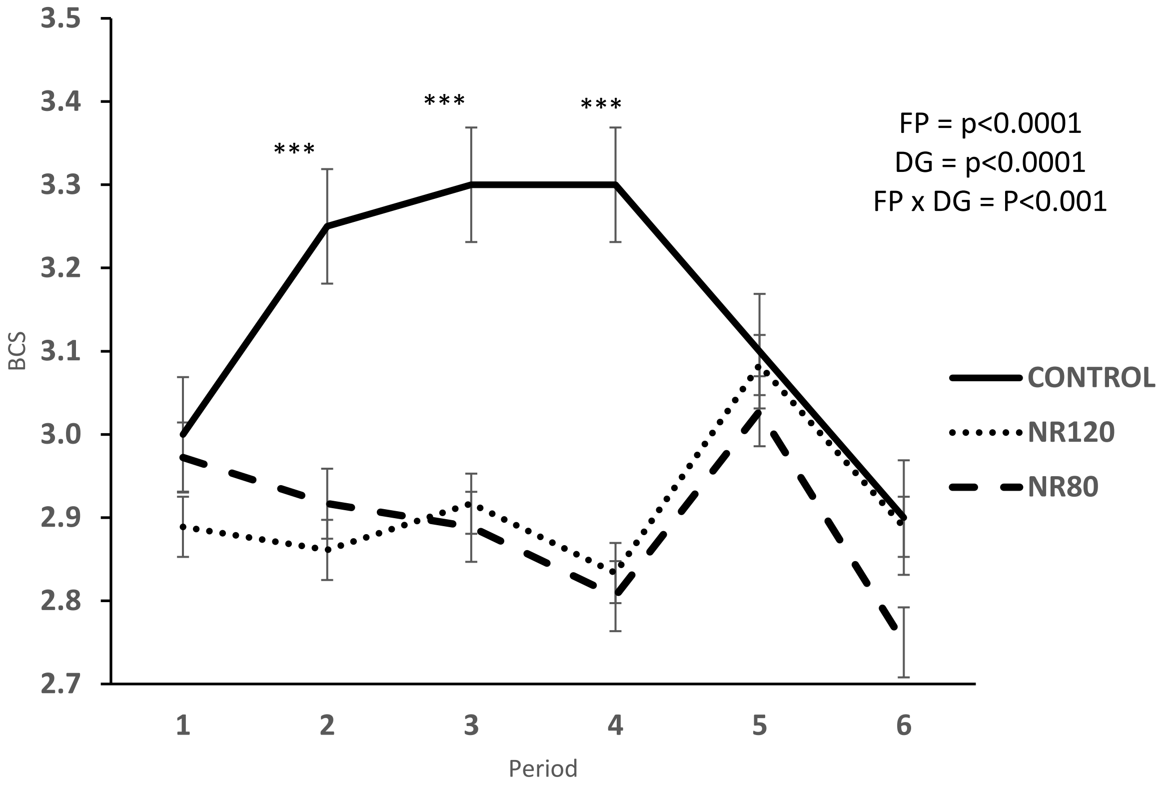


Figure 3.4a. Body condition score (BCS) of heifers (n=24) at varying periods including pre, during and post pregnancy. Period 1 = Before artificial insemination, Period 2 = Day of artificial insemination, Period 3 = First pregnancy confirmation (d 28 – 35), Period 4 = Second pregnancy confirmation (d 55-65), Period 5 = At calving, Period 6 = 30 days after calving. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean.

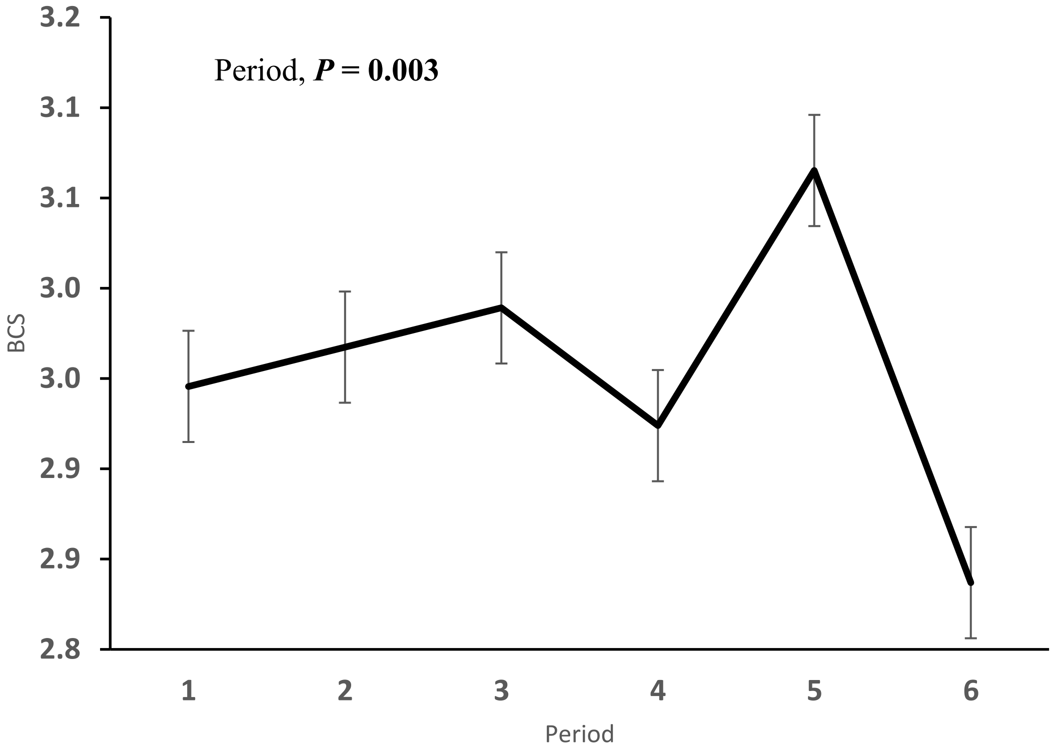


Figure 3.4b. Body condition score (BCS) of all heifers (n=24) regardless of nutritional group at varying periods including pre, during and post pregnancy. Period 1 = Before artificial insemination, Period 2 = Day of artificial insemination, Period 3 = First pregnancy confirmation (d 28 – 35), Period 4 = Second pregnancy confirmation (d 55-65), Period 5 = At calving, Period 6 = 30 days after calving. Error bars represent standard error of mean

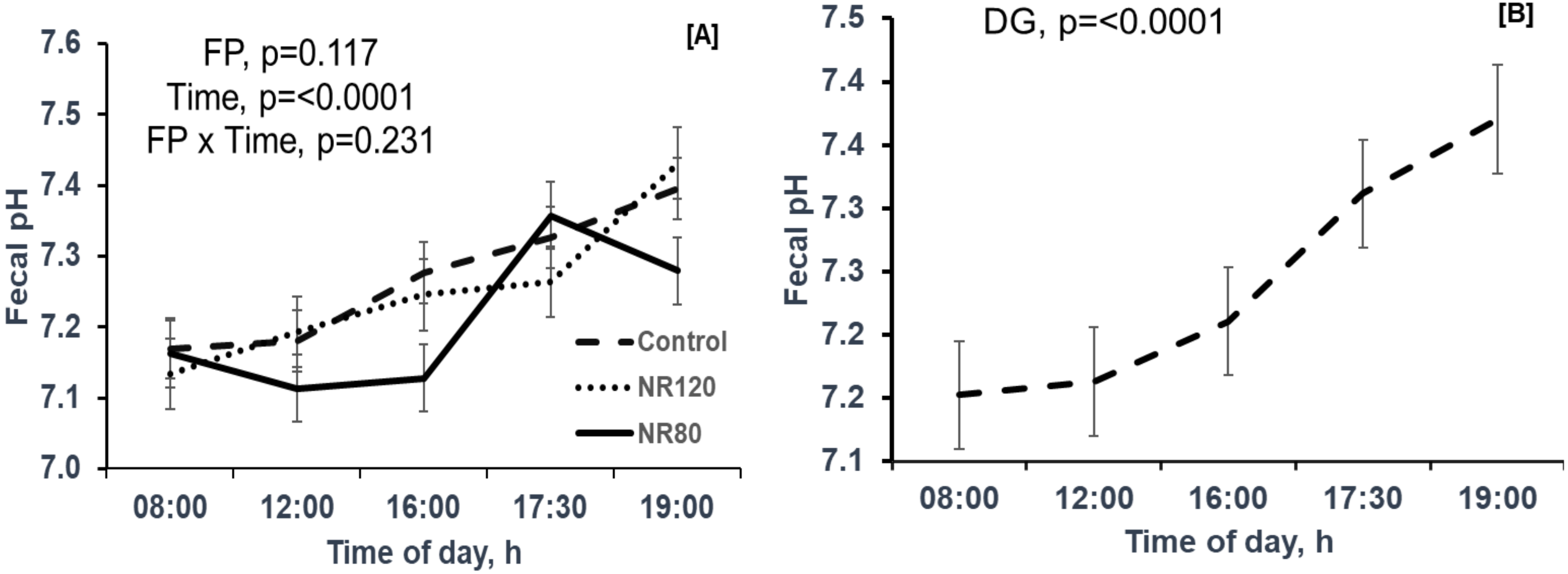


Figure 3.5a-b. Mean hourly variation of fecal pH in pregnant heifers (n=24) throughout pregnancy. Fecal pH was assessed during each time point of fecal collection at the different days of gestation with a mean daily pH of 7.2. DG = Day of gestation. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean.

(a)

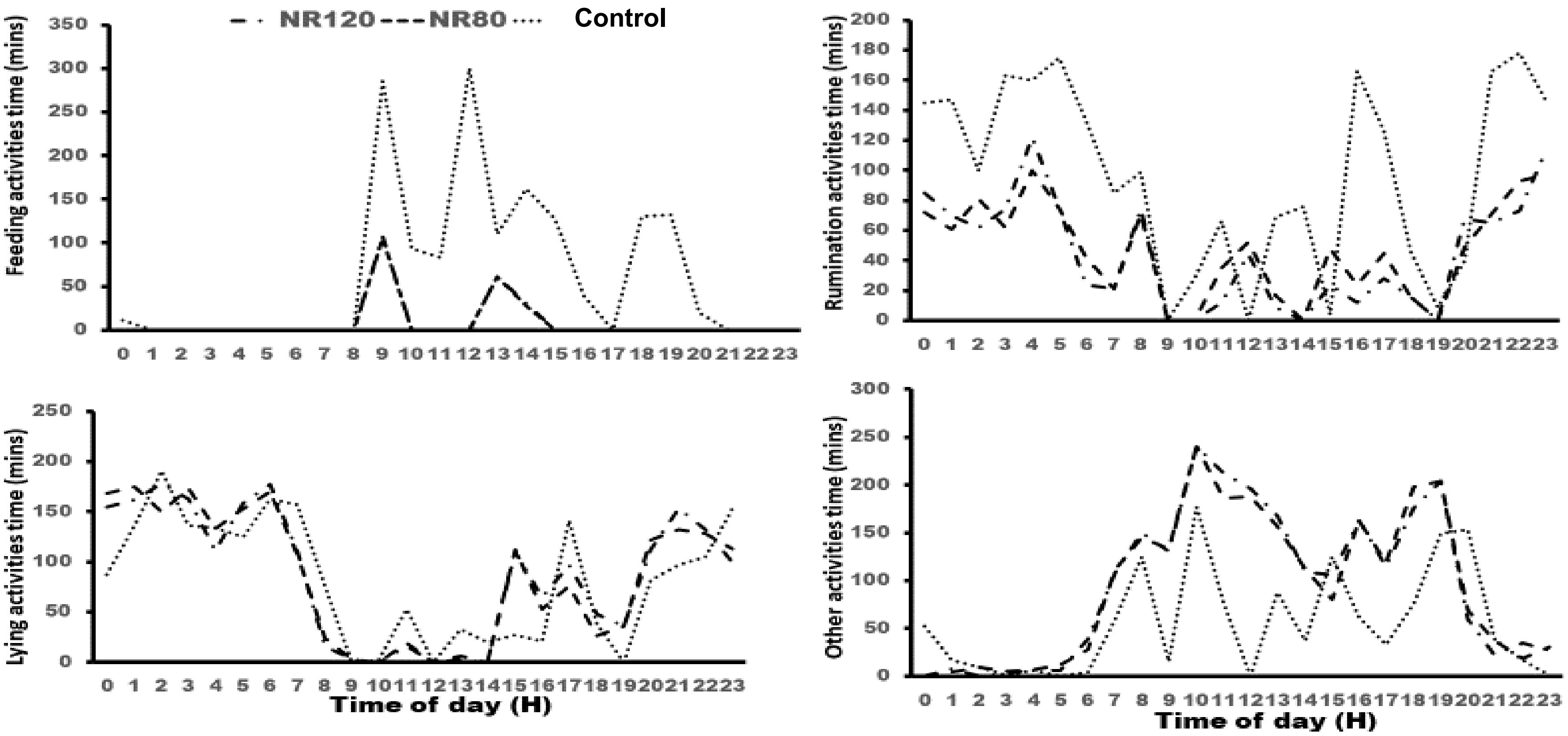


Figure 3.6a. Hourly behavioural outcomes of gravid heifers (n=13) in the first trimester (d 65) of pregnancy. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

(b)

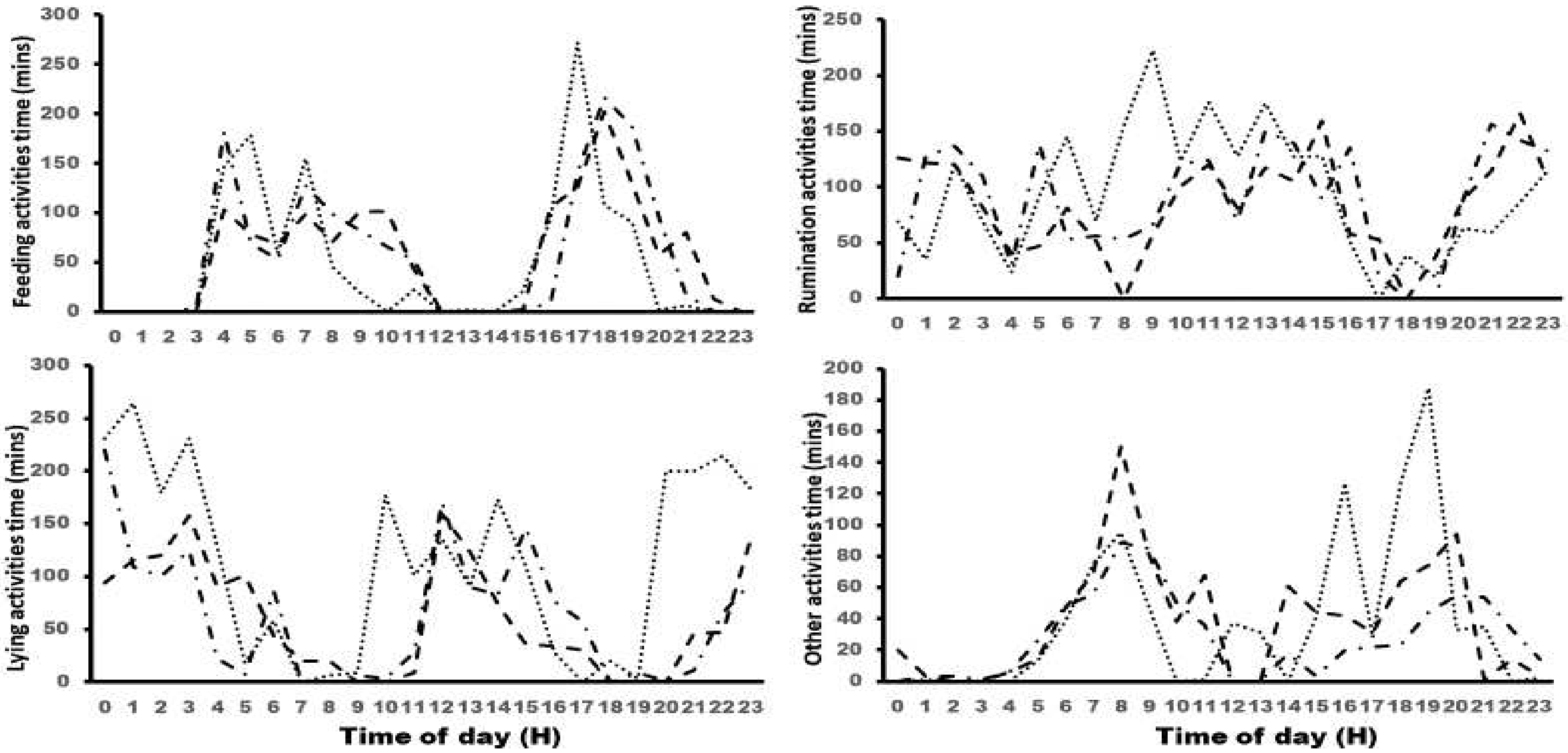


Figure 3.6b. Hourly behavioural outcomes of gravid heifers (n=13) in the Second trimester (d 170) of pregnancy. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

(c)

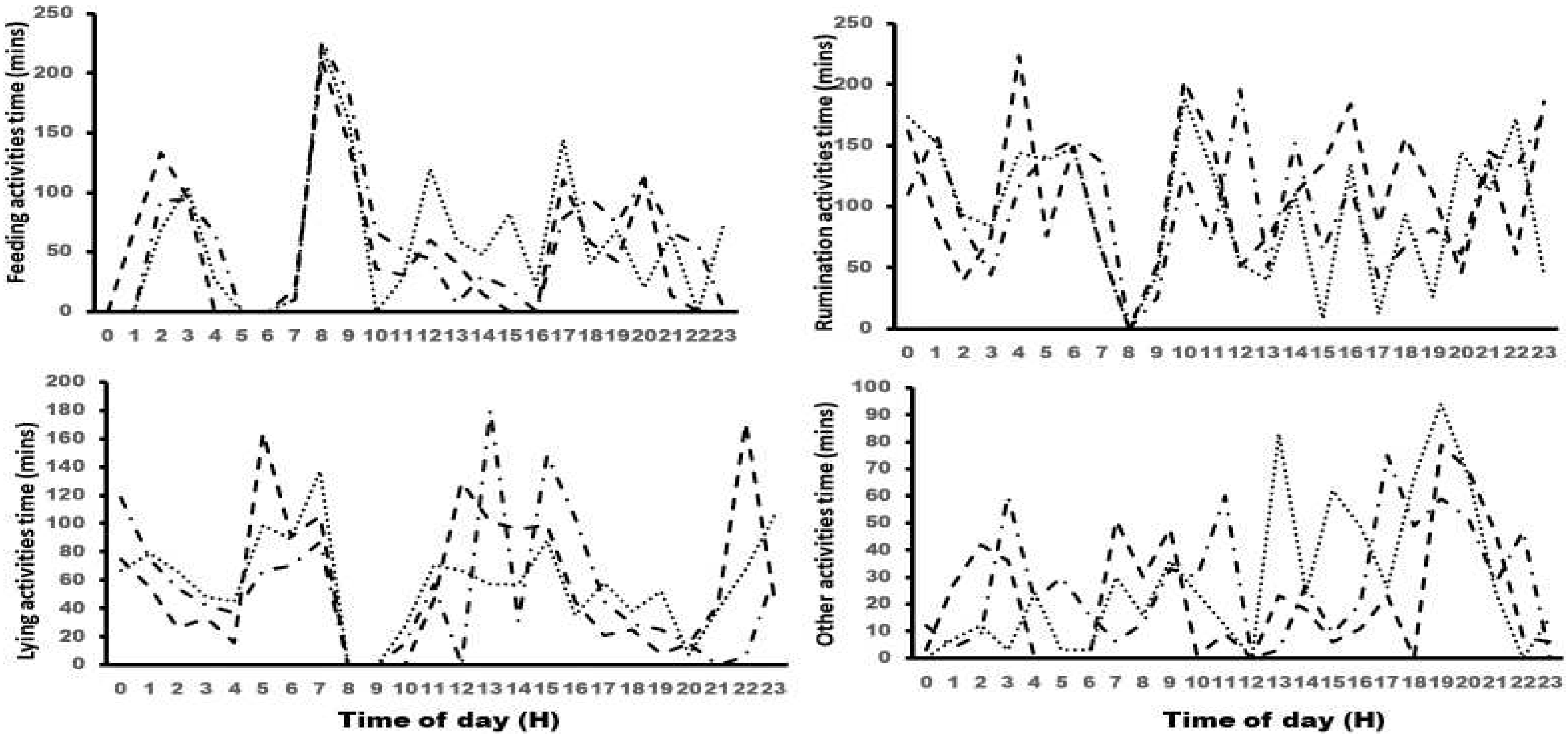


Figure 3.6c. Hourly behavioural outcomes of gravid heifers (n=13) in the third trimester (d 270) of pregnancy. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation

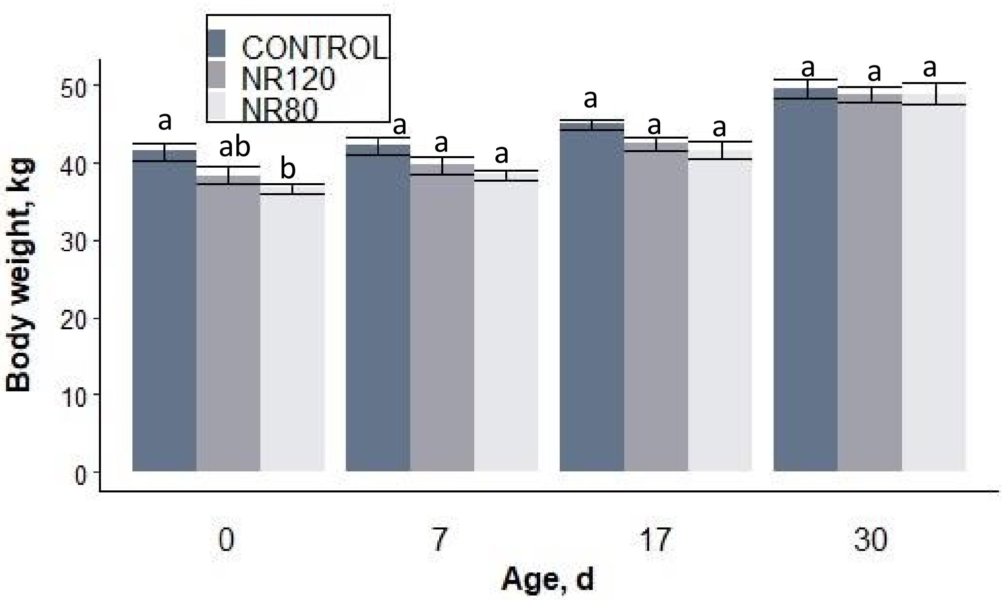


Figure 3.7. Body weight of female calves (n = 22) from birth to d 30 postnatal. Day 0 represents the birth weight of calves. Calves were born to mothers fed either NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation, NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of the mean.

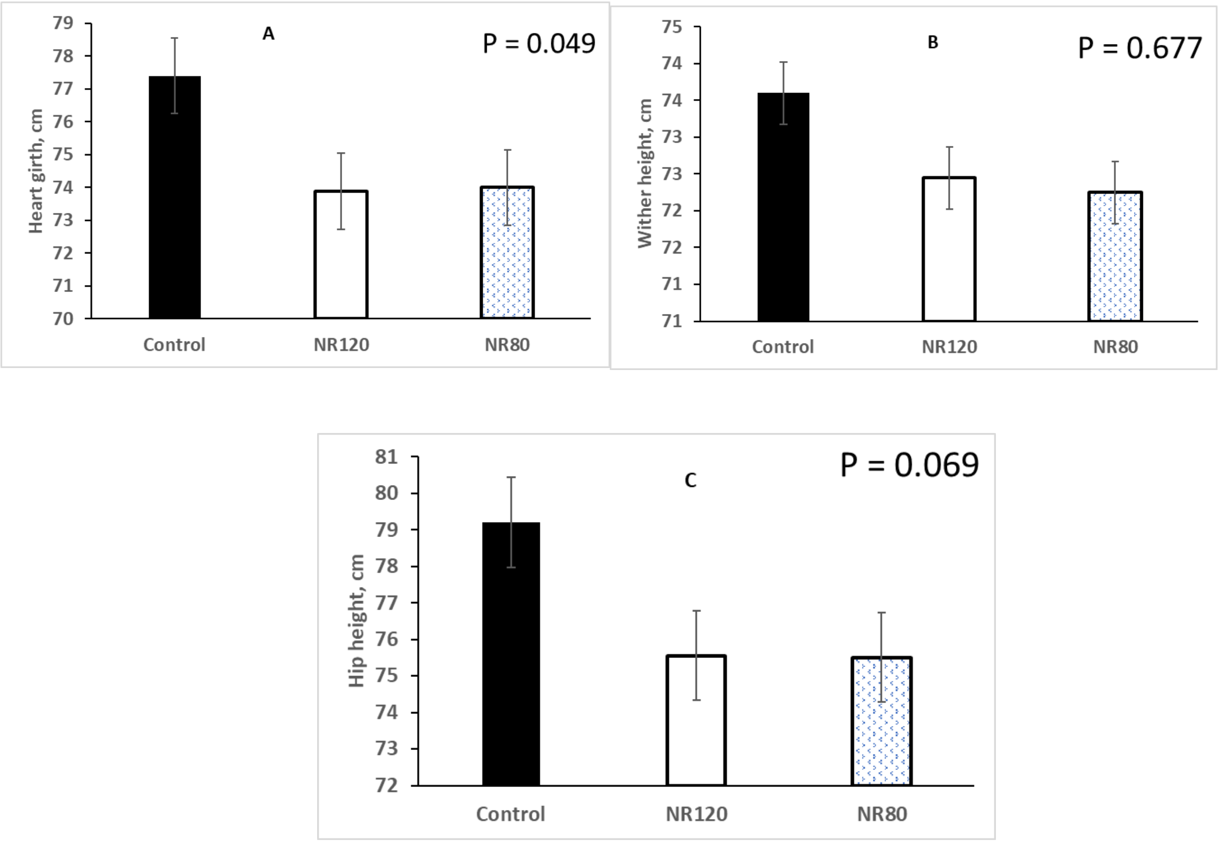


Figure 3.8a-c. Calves body linear measurements (n=22) at birth. Calves were born from mother fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

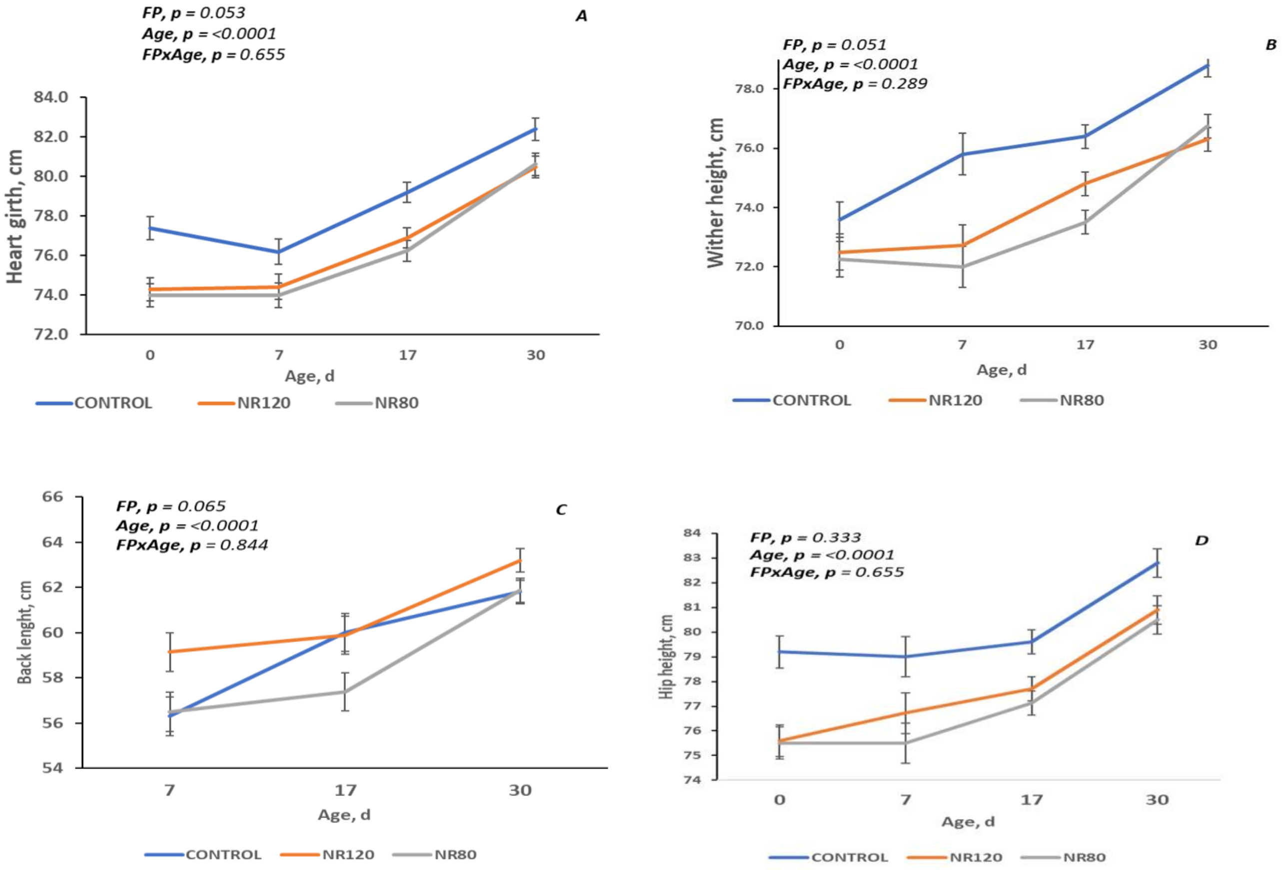


Figure 3.9a-d. Calves body linear measurements (n=22) within the first one month of life. Calves were born from mother fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

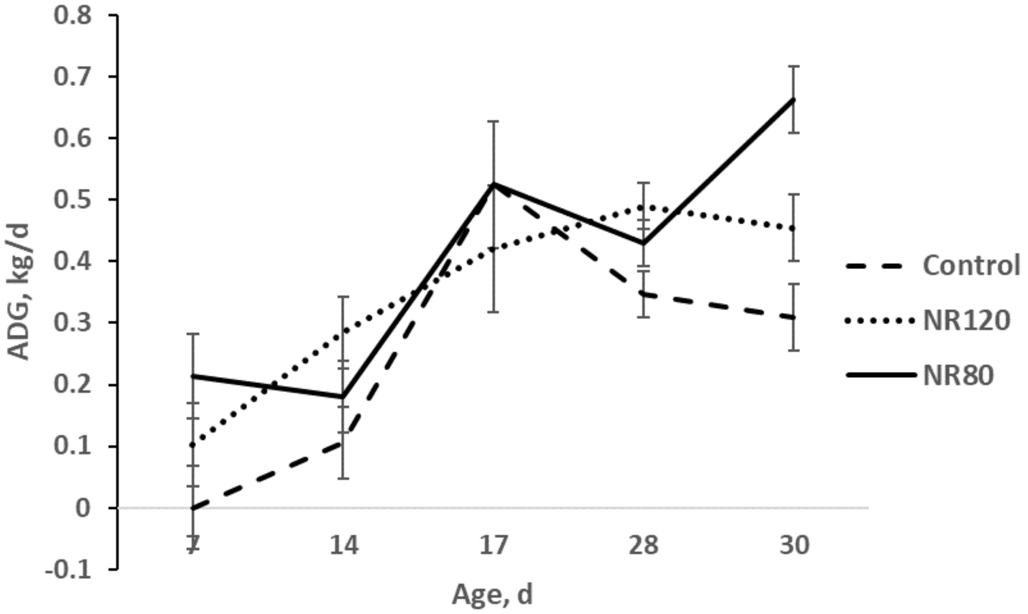


Figure 3.10. Calves average daily gain (n=22) within the first one month of life. Calves were born from mother fed either; NR80=0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120=0.6 M until day 120 of gestation and Control=ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean.

REFERENCES

Abuelo, A. 2020. Symposium review: Late-gestation maternal factors affecting the health and development of dairy calves. Journal of Dairy Science, 103(4), Pp. 3882-3893, ISSN 0022-0302, https://doi.org/10.3168/jds.2019-17278.

Alharthi, A.S., Coleman, D.N., Alhidary, I.A., Abdelrahman, M.M., Trevisi, E. and Loor, J.J. 2021. Maternal body condition during late pregnancy is associated with in utero development and neonatal growth of Holstein calves. Journal of Animal Science and Biotechnology, 12:44. https://doi.org/10.1186/s40104-021-00566-2.

AOAC International. 2000. Official Method of Analysis. 17th ed. AOAC International, Arlington, VA.

AOAC International. 2005. Official Method of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

AOAC. 1995. Official Methods of Analysis of Association of Official Analytical Chemist. AOAC International. Virginia USA.

Barker D J P. 2004. Developmental origins of well-being. Philos. Trans. Royal Soc. London. 359:1359–1366. https://doi:10.1098/rstb.2004.1518.

Barker, D. J. P. 1992. Fetal and Infant Origins of Adult Disease. London: BMJ Publishing Group.

Bazeley, K.J., Barrett, D.C., Williams, P.D. and Reyher, K.K. 2016. Measuring the growth rate of UK dairy heifers to improve future productivity. Veterinary Journal, 212: 9-14. https://doi.org/10.1016/j.tvjl.2015.10.043.

Beauchemin, K.A. 2018. Invited review: Current perspectives on eating and rumination activity in dairy cows. Journal of Dairy Science, 101(6): 4762-4784. doi: 10.3168/jds.2017-13706.

Beharka, A., Cochran, B., Harmon, D. and Avery, T. 1988. The influence of stage of pregnancy on digestion characteristics of beef cows. Kansas State Univ. Prog. Rep. 539:13–15.

Boterman, E., and Bucholtz. H. 2005. Feeding practices of high-producing herds in Michigan Proceedings of the Tri-State Dairy Nutrition Conference, The Ohio State University, Columbus (2005), pp. 113-129.

Caton, J. S., Crouse, M. S., Reynolds, L. P., Neville, T. L., Dahlen, C. R., Ward, A. K., and Swanson, K. C. 2019. Maternal nutrition and programming of offspring energy requirements. Translational animal science, *3*(3): 976–990. https://doi.org/10.1093/tas/txy127.

Chebel, R., Mendonça, L.G.D. and Baruselli, P.S. 2018. Association between body condition score change during the dry period and postpartum health and performance. Journal of Dairy Science. 101(5): 4595-4614. https://doi.org/10.3168/jds.2017-13732.

Clark, J. H., Olson, K.C., Schmidt, T.B., Linville, M.L., Alkire, D.O., Meyer, D.L., Rentfrow, G.K., Carr, C.C. and Berg, E.P. 2007. Effects of dry matter intake restriction on diet digestion, energy partitioning, nutrient retention, and ruminal fermentation by beef steers. J. Anim. Sci. 85:3383–3390. http://dx.doi.org/10.2527/jas.2006-741.

Cook, N.B., Bennett, T. B. and Nordlund, K. V. 2004. Effect of Free Stall Surface on Daily Activity Patterns in Dairy Cows with Relevance to Lameness Prevalence. Journal of Dairy Science. 87:2912–2922.

Copping, K. J., Ruiz-Diaz, M. D., Rutland, C. S., Mongan, N. P., Callaghan, M. J., McMillen, I. C., Rodgers, R. J. and Perry, V. E. A. 2018. Periconception and first trimester diet modifies reproductive development in bulls. Reprod. Fertil. Dev. 30, 703–720. doi:10.1071/RD17102.

Costa, T.C., Dutra, L.L., Mendes, T.A.d.O., dos Santos, M.M., Veroneze, R., Gionbelli, M.P. and Duarte, M.d.S. 2022. Impact of Maternal Feed Restriction at Different Stages of Gestation on the Proteomic Profile of the Newborn Skeletal Muscle. Animals, 12, 1011. https:// doi.org/10.3390/ani1208101.

Dado-Senn, B., Field, S.L., Davidson, B.D., Casarotto, L.T., Marrero, M.G., Ouellet, V., Cunha, F., Sacher, M.A., Rice, C.L., Maunsell, F.P., Dahl, G.E. and Laporta, J. 2021. Late-Gestation in utero Heat Stress Limits Dairy Heifer Early-Life Growth and Organ Development. Frontiers in Animal Science. 2:750390. doi: 10.3389/fanim.2021.750390.

Dado-Senn, B., Laporta, J. and Dahl, G. E. 2020a. Carry over effects of late gestational heat stress on dairy cattle progeny. Theriogenology 154, 17–23. doi: 10.1016/j.theriogenology.2020.05.012.

Dann, H. M., Varga, G. A. and Putnam, D. E. 1999. Improving Energy Supply to Late Gestation and Early Postpartum Dairy Cows. Journal of Dairy Science. 82:1765–1778.

Das, K.S. and Das, N. 2007. Feeding Behavior of Pregnant Dairy Heifers during Last Trimester under Loose Housing System. Asian-Austrain Journal of Animal Science. 20(9):1402-1406.

DeVries, T.J., von Keyserlingk, M.A.G., Weary, D.M. and Beauchemin, K.A. 2003. Technical Note: Validation of a System for Monitoring Feeding Behavior of Dairy Cows. Journal of Dairy Science, 86:3571–3574.

Diniz, W. J. S., Crouse, M. S., Cushman, R. A., McLean, K. J., Caton, J. S. and Dahlen, C. R. 2021a. Cerebrum, liver, and muscle regulatory networks uncover maternal nutrition effects in developmental programming of beef cattle during early pregnancy. Sciencia. Rep. 11:2271. doi: 10.1038/s41598-021-82156-w.

Dirksen, G. 1986. Ruminal acidosis complex—New observations and experiences. A review. Tierarztl. Prax. 14:23–33.

Duarte, M.S., Gionbelli, M.P., Paulino, P.V.R., Seraoc, N.V.L., Martins, T.S., Totaro, P.I.S., Neves, C.A., Filho, S.C.V., Dodson, M.V., Zhu, M. and Du, M. 2013. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. Livestock Science, 153 (2013), pp. 60-65.

Duplessis, M., Cue, R.I., Santschi, D.E., Lefebvre, D.M. and Lacroix, R. 2015. Weight, height, and relative-reliability indicators as a management tool for reducing age at first breeding and calving of dairy heifers. Journal of Dairy Science. 98(3):2063-73. doi: 10.3168/jds.2014-8279.

Erickson, P.S., Anderson, J.L., Kalscheur, K.F., Lascano, G.J., Akins, M.S. and Heinrichs, A.J. 2020. Symposium review: Strategies to improve the efficiency and profitability of heifer raising. Journal of Dairy Science, 103(6): P. 5700-5708, ISSN 0022-0302. https://doi.org/10.3168/jds.2019-17419.

Erickson, P.S., Anderson, J.L., Kalscheur, K.F., Lascano, G.J., Akins, M.S. and Heinrichs, A.J. 2020. Symposium review: Strategies to improve the efficiency and profitability of heifer raising. Journal of Dairy Science. 103(6):5700-5708. https://doi.org/10.3168/jds.2019-17419.

Ferrell, C. L., Garrett, W. N. and Hinman, N. 1976b. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. Journal of Animal Science. 42, 1477–1489. https://doi .org/10.2527/jas1976.4261477x.

Forbes, J.M. 2009. The physical relationships of the abdominal organs in the pregnant ewe. Journal of Agricultural Science 70, 171–177.

Ford, S.P., Hess, B.W., Schwope, M.M., Nijland, M.J., Gilbert, J.S., and Vonnahme, K.A. 2007. Maternal Undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. Journal of Animal Science. 85:1285-1294. Doi:10.2527/jas.20045-624.

Franchi, G.A., Herskin, M.S. and Jensen, M.B. 2019. Dairy cows fed a low energy diet before dry-off show signs of hunger despite *ad libitum* access. *Sci Rep* **9,** 16159 https://doi.org/10.1038/s41598-019-51866-7.

French, P. D. 2006. Dry matter intake and blood parameters of nonlactating Holstein and Jersey cows in late gestation. Journal of Dairy Science. 89:1057–1061. http://dx.doi.org/10.3168/jds.S0022-0302(06)72173-7.

Fustini, M., A. J. Heinrichs, A. Palmonari, and A. Formigoni. 2016. Farm characteristics and total mixed ration particle size issues on Parmigiano Reggiano farms in Northern Italy. Anim. Sci. 32:869–873.

Gallo, A., Giuberti, G., Bruschi, S., Fortunati, P. and Masoero, F. Technical note: Relationship between in situ NDF degradability and enzymatic NDF hydrolysis in forages, non forage fibrous feeds, and crop residues. Journal of Animal Science. 2017, 95, 4172–4180.

Gonzalez-Recio, O., Ugarte, E. and Bach, A. 2012. Trans-generational effect of maternal lactation during pregnancy: A Holstein cow model. PLoS One, 7 p. e51816. 10.1371/journal.pone.0051816.

Greter, A.M., DeVries, T.J. and von Keyserlingk, M.A.G. 2008. Nutrient intake and feeding behavior of growing dairy heifers: effects of dietary dilution. Journal of Dairy Science: 91: pp. 2786-2795. https://doi.org/10.3168/jds.2008-1052.

Greter, A.M., Leslie, K.E., Mason, G.J., McBride, B.W. and DeVries, T.J. 2011. Effect of feed delivery method on the behavior and growth of dairy heifers. Journal of Dairy Science. 93 (2010), pp. 1668-1676. https://doi.org/10.3168/jds.2009-2844.

Hafez, E.S.E. and Bouissou, M.F. 1975. The behaviour of cattle. in: Hafez E.S.E. The Behaviour of Domestic Animals. 3rd ed. Bailliere Tindall, London, UK: 203-245.

Heinrichs, A.J., Heinrichs, B.S., Cavallini, D., Fustini, M. and Formigoni, A. 2021. Limiting total mixed ration availability alters eating and rumination patterns of lactating dairy cows. JDS Communications, 2(4), Pp. 186-190. https://doi.org/10.3168/jdsc.2020-0074.

Hoffman, P.C., Simson, C.R. and Wattiaux, M. 2007. Limit feeding of gravid Holstein heifers: effect on growth, manure nutrient excretion, and subsequent early lactation performance. Journal of Dairy Science, 90(2):946-54. doi: 10.3168/jds.S0022-0302(07)71578-3. PMID: 17235171.

Hofmman, P.C. 2017. Feeding management of the dairy heifer from 4 months to calving DK Beede (Ed.), Large Dairy Herd Management, American Dairy Science Association, Champaign (2017), pp. 431-44.

Hummel, G., Woodruff, K., Austin, K., Knuth, R., Lake, S. and Cunningham-Hollinger, H. 2021. Gestation Maternal Feed Restriction Decreases Microbial Diversity of the Placenta While Mineral Supplementation Improves Richness of the Fetal Gut Microbiome in Cattle. Animals, 11, 2219. https:// doi.org/10.3390/ani11082219.

Hyde, R.M., Green, M.J., Hudson, C. and Down, P.M. 2022. Improving growth rates in preweaning calves on dairy farms: A randomized controlled trial. Journal of Dairy Science, 105(1), 782-792. https://doi.org/10.3168/jds.2021-20947.

Hyde, R.M., Green, M., Hudson, C. and Down, P**.** 2021. Factors associated with daily weight gain in preweaned calves on dairy farms**.** Prev. Vet. Med., 190 (2021), Article 105320. https://doi.org/10.1016/j.prevetmed.2021.105320.

Kmicikewycz, A. D., Harvatine, K.J. and Heinrichs, A.J. 2015. Effects of corn silage particle size, supplemental hay, and forage to- concentrate ratio on rumen pH, feed preference, and milk fat profile of dairy cattle. Journal of Dairy Science. 98:4850–4868.

Lammers, B.P., Heinrichs, A.J. and Kensinjer, R.S. 1999. The effects of accelerated growth rates and estrogen implants in prepubertal Holstein heifers on estimates of mammary development and subsequent reproduction and milk production. Journal of Dairy Science. 82: 1753-1764.

Laporta, J., Fabris, T. F., Skibiel, A. L., Powell, J. L., Hayen, M. J., Horvath, K., Miller-Cushon, E.K., and Dahl, G.E. 2017. In utero exposure to heat stress during late gestation has prolonged effects on the activity patterns and growth of dairy calves. Journal of Dairy Science. 100, 2976–2984. doi: 10.3168/jds.2016-11993.

Laporta, J., Ferreira, F. C., Ouellet, V., Dado-Senn, B., Almeida, A. K., De Vries, A. and Dahl, G.E. 2020. Late-gestation heat stress impairs daughter and granddaughter lifetime performance. Journal of Dairy Science. 103, 7555–7568. doi: 10.3168/jds.2020- 18154.

Licitra, G., Hernandez, T.M. and Van Soest, P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358.

Lindström, T and Redbo, I.I. 2000. Effect of feeding duration and rumen fill on behaviour in dairy cows. Appl Anim Behav Sci. 70(2):83-97. doi: 10.1016/s0168-1591(00)00148-9. PMID: 11080553.

Linton J.A. and Allen, M.S. 2007. Nutrient Demand Affects Ruminal Digestion Responses to a Change in Dietary Forage Concentration. Journal of Dairy Science, 90(10): 4770-4779. https://doi.org/10.3168/jds.2007-0100.

Long, N.M., Prado-Cooper, M.J., Krehbiel, C.R., DeSilva, U. and Wettemann, R.P. 2010. Effects of nutrient restriction of bovine dams during early gestation on postnatal growth, carcass and organ characteristics, and gene expression in adipose tissue and muscle. Journal of Animal Science. 88(10):3251-61

Luan, S., Cowles, K., Murphy, M.R. and Cardoso, F.C. 2016. Effect of a grain challenge on ruminal, urine, and fecal pH, apparent total tract starch digestibility, and milk composition of Holstein and Jersey cows. Journal of Dairy Science, 99:2190–2200.

Macdonald, K.A., Penno, J.W., Bryant, J.M. and Roche, J.R. 2005. Effect of Feeding Level Pre- and Post-Puberty and Body Weight at First Calving on Growth, Milk Production, and Fertility in Grazing Dairy Cows. Journal of Dairy Science, 88:3363–3375.

McCoski, S., Bradbery, A., Marques, R., Posbergh, C. and Sanford, C. 2021. Maternal Nutrition and Developmental Programming of Male Progeny. Animals: an open access journal from MDPI, 11(8), 2216. https://doi.org/10.3390/ani11082216.

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

Micke, G. C., Sullivan, T.M., Gatford, K. L., Owens, J. A., and Perry, V. E. A. 2010. Nutrient intake in the bovine during early and mid-gestation causes sex-specific changes in progeny plasma IGF-1, liveweight, height and carcass traits. Animal Reproduction Science. 121, 208–217. doi: 10.1016/j.anireprosci.2010.05.017.

Miedema, H.M., Cockram, M.S., Dwyer, C.M. and Macrae, A.I. 2011. Changes in the behavior of dairy cows during the 24 h before normal calving compared with behavior during late pregnancy. Appl. Anim. Behavior Science, 132, pp. 8-14.

Mishra, S., Kumari, K. and Dubey, A. 2016. Body condition scoring of dairy cattle: A review, research and reviews. Journal of Veterinary Sciences. 2(1). Available from: http://www.rroij.com/open-access/body-condition-scoring-of-dairy-cattle-a-review-pdf.

Mossa, F., Carter, F., Walsh, S.W., Kenny, D.A., Smith, G.W., Ireland, J.L., Hildebrandt, T.B., Lonergan, P., Ireland, J.J. and Evans, A.C. 2013. Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. Biol. Reprod. 88:92. doi:10.1095/ biolreprod.112.107235.

Murphy, T. A., Loerch, S.C. and Smith, F.E. 1994. Effects of feeding high-concentrate diets at restricted intakes on digestibility and nitrogen metabolism in growing lambs. Journal of Animal Science. 72:1583–1590.

Murtaugh, P.A. 2014. In defense of P values. Ecological Society of America. https://doi.org/10.1890/13-0590.1.

National Academies of Sciences, Engineering, and Medicine, NASEM (2016). Nutrient Requirements of Beef Cattle: Eighth Revised Edition. The National Academies Press; Washington, DC, USA: 2016. p. 494.

Noya, A., Casasús, I., Ferrer, J. and Sanz, A. 2019. Long-term effects of maternal subnutrition in early pregnancy on cow-calf performance, immunological and physiological profiles during the next lactation. Animal, 9: 936.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Ohnstad, I. 2013. Body condition scoring in dairy cattle: Monitoring health to improve milk yield and fertility. Livestock. 18(3). DOI:10.12968/live.2013.18.3.70.

Paradis, F., Wood, K.M., Swanson, K.C. Miller, S.P., McBride, B.W. and Fitzsimmons, C. 2017. Maternal nutrient restriction in mid-to-late gestation influences fetal mRNA expression in muscle tissues in beef cattle. BMC Genomics 18, 632. https://doi.org/10.1186/s12864-017-4051-5.

Park, C. S., Choi, Y.J., Erickson, G.M. and Marx, G.D. 1987. Effect of compensatory growth on regulation of growth and lactation: response of dairy heifers to a stair-step growth pattern. J. Anim. Sci. 64:1751.

Pereira, J. M. V., Marcondes, M. I., Filho, S. D. V. H., Caton, J., Sguizzato, A. L. L., Silva, A. L., da Silva, J. T., Moraes, V. C. L., Gomes, L. F. and Rotta, P. P. 2020. Digestive parameters during gestation of Holstein heifers: Digestion during pregnancy. Livestock Science, 242, 104325. https://doi.org/10.1016/j.livsci.2020.104325.

Pérez-Báez, J., Risco, C.A., Chebel, R.C., Gomes, G.C., Greco, L.F., Tao, S., Thompson, I.M., do Amaral, B.C., Zenobi, M.G., Martinez, N., Staples, C.R., Dahl, G.E., Hernández, J.A., Santos, J.E.P. and Galvão, K.N. 2019. Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part II. Ketosis and clinical mastitis. Journal of Dairy Science, 102(10): 9151-9164.

Pino, F., Mitchell, L.K., Jones, C.M. and Heinrichs, A.J. 2018. Comparison of diet digestibility, rumen fermentation, rumen rate of passage, and feed efficiency in dairy heifers fed ad-libitum versus precision diets with low- and high-quality forages. J. Appl. Anim. Res., 46 (2018):1296-1306.

R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats (10th), Elsevier Publishing, New York, NY (2007)

Radunz, A. E., Fluharty, F. L., Relling, A. E., Felix, T. L., Shoup, L. M. and Zerby, H. N. 2012. Prepartum dietary energy source fed to beef cows: II. effects on progeny postnatal growth, glucose tolerance, and carcass composition. Journal of Animal Science. 90, 4962–4974. doi: 10.2527/jas.2012-5098.

Ramanoon, S.Z., Sadiq, M.B., Mansor, R., Syed-Hussain, S.S., Mossadeq, W.M.S. 2018. The Impact of Lameness on Dairy Cattle Welfare: Growing Need for Objective Methods of Detecting Lame Cows and Assessment of Associated Pain. Animal Welfare.

Reynolds, C.K., Dürst, B., Lupoli, B., Humphries, D.J. and Beever, D.E. 2004. Visceral tissue mass and rumen volume in dairy cows during the transition from late gestation to early lactation. Journal of Dairy Science, 87: 961–971.

Reynolds, L P. and Caton, J.S. 2012. Role of the pre- and post-natal environment in developmental programming of health and productivity. Mol. Cell. Endocrinol. 354:54–59. https://doi:10.1016/j.mce.2011.11.013.

Rodríguez-Sánchez, J.A., Sanz, A., Ferrer, J., Ripoll, G. and Casasús, I. 2016. First calving performance and physiological profiles of 2-year-old beef heifers according to their pre-breeding growth. Canadian Journal of Animal Science. **97**(3): 488-498. https://doi.org/10.1139/cjas-2016-0207.

Rotta, P.P., Valadares Filho, S.C., Gionbelli, T.R.S., Costa e Silva, L.F., Engle, T.E., Marcondes, M.I., Machado, F.S., Villadiego, F.A.C. and Silva, L.H.R. 2015. Effects of day of gestation and feeding regimen in Holstein × Gyr cows: I. Apparent total-tract digestibility, nitrogen balance, and fat deposition. Journal of Dairy Science. 98(5):3197-3210. https://doi.org/10.3168/jds.2014-8280.

Savory, C.J., Maros, K. and Rutter, S.M. 1993. Assessment of hunger in growing broiler breeders in relation to a commercial restricted feeding program. Animal Welfare; 2: 131-152.

Shivley, C.B., Lombard, J.E. and Urie, N.J. 2018. Pre-weaned heifer management on US dairy operations: Part VI. Factors associated with average daily gain in pre-weaned dairy heifer calves. Journal of Dairy Science, 101: 9245-9258.

Silva, F.A.S., Valadares Filho, S.C., Rennó, L.N., D. Zanetti, L. F. Costa e Silva, L. A. Godoi, J. M. P. Vieira, A. C. B. Menezes, P. Pucetti, P. P. Rotta, P.P. 2017. Energy and protein requirements for growth of Holstein × Gyr heifers. Journal of Animal Physiology and Animal Nutrition. 102(1):82-93.https://doi.org/10.1111/jpn.12661

Soberon, F., Raffrenato, E. and Everett, R. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. Journal of Dairy Science, 95:783-793.

Souissi, W. and Bouraoui, R. 2019. Relationship between Body Condition Score, Milk Yield, Reproduction, and Biochemical Parameters in Dairy Cows. In (Ed.), Lactation in Farm Animals - Biology, Physiological Basis, Nutritional Requirements, and Modelization. IntechOpen. https://doi.org/10.5772/intechopen.85343

Taylor, R., LeMaster, C., Mangrum, K., Ricks, R., and Long, N. 2018. Effects of maternal nutrient restriction during early or mid-gestation without realimentation on maternal physiology and foetal growth and development in beef cattle. Animal, 12(2): 312-321. doi:10.1017/S175173111700163X

Tedeschi L.O. and Fox, D.G. 2020. Ruminant Nutrition System, Vol. I — An Applied Model for Predicting Nutrient Requirement and Feed Utilization in Ruminants. ISBN: 978-1-97507-701-3.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74: 3583–3597.

Vanegas, J., Overton, M., Berry, S.L. and Sischo, W.M. 2006. Effect of rubber flooring on claw health in lactating dairy cows housed in free-stall barns. Journal of Dairy Science. 89: 4251- 4258.

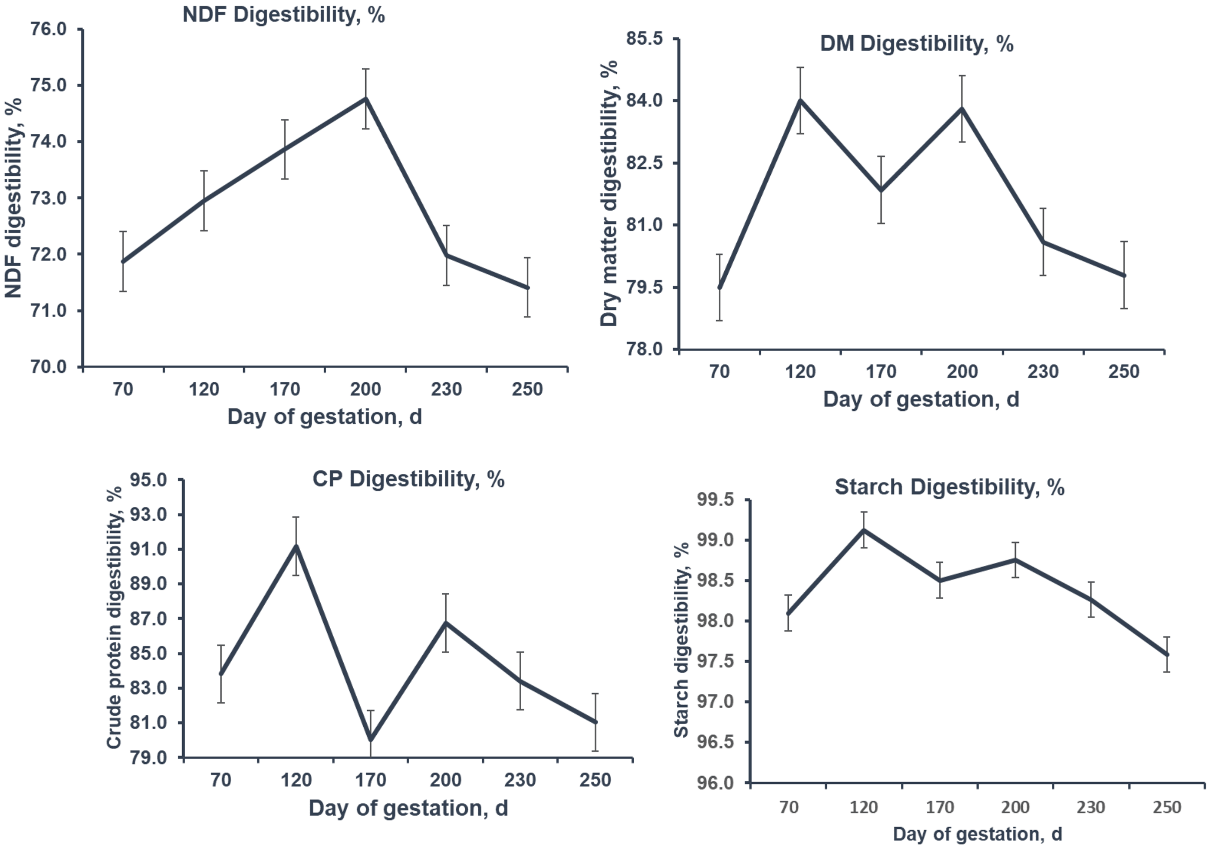
Weiss, W. P. 1999. Energy prediction equations for ruminant feeds. Pages 176–185 in Proc. 61st Cornell Nutrition Conference for Feed Manufacturers, Ithaca, NY. Cornell University, Ithaca, NY.

Williams, K.T., Weigel, K.A., Coblentz, W.K., Esser, N.M., Schlesser, H., Hoffman, P.C., Ogden, R., and Akins, M.S. 2020. Effect of diet energy level and genomic residual feed intake on bred Holstein dairy heifer growth and feed efficiency. Journal of Dairy Science, 105(3): https://doi.org/10.3168/jds.2020-19982.

Yang, W.Z. and Beauchemin, K.A. 2007. Altering physically effective fiber intake through forage proportion and particle length: Chewing and ruminal pH. Journal of Dairy Science. 90(6):2826–2838.

Zebeli, Q. J., Mansman, D., Steingass, H. and Ametaj, B.N. 2009. Balancing diets for physically effective fibre and ruminally degradable starch: A key to lower the risk of sub-acute rumen acidosis and improve productivity of dairy cattle. Livestock Science. 127:1–10.

APPENDIX 1



Appendix 3.1. Pooled apparent total tract digestibility of heifers (n=24) during pregnancy. error bars represent standard error of mean.

CHAPTER 4

Effects of early-gestation maternal energy restriction in heifers on solid feed intake, behavioural patterns, growth performance and apparent total tract digestibility of pre-weaned dairy calves

ABSTRACT

Altering maternal nutrition during gestation have been well documented to regulate fetal development and shape offspring postnatal performance through altered organ and tissue development. This study aimed to investigate the critical variables on feeding replacement dairy heifers a limited amount of their maintenance energy requirements in early gestation on female offspring growth performance, feed intake, behavior, and apparent total tract digestibility during their preweaning phase. Briefly, Holstein Friesian heifers (n=28; BW±SD; 371.57±42.14, age±SD; 15.7±1.08 months) were divided into three groups based on body weight and age and, from 11 days before insemination were individually fed as following: (i) 0.6 M of their maintenance energy requirements (M) until day 80 of gestation (nutrient restricted NR80, n = 11), (ii) 0.6 M until day 120 of gestation (NR120, n=11), and (iii) ad libitum (1.8 M) until day 120 of gestation (Control, n=6). Heifers were group fed once the differential feeding period ended, with ad libitum access to feed until calving. Twenty-two single female calves were retained after calving. All calves received the same management and diet until weaning (age±SD; 69±7 days). Daily individual intake was recorded, and body weight and body dimension measurements were monitored every fourth night until weaning. A 12-hour diurnal behavioural observation was carried out at postnatal weeks 3 and 4, while fecal collection to estimate apparent total tract nutrient digestibility was performed during the last 3 days to weaning. A mixed model with repeated measures was used for data analysis (growth, and intake) in R. Excess heifer nutrition during early-pregnancy influenced calves birth weight (P=0.024), and heart girth (P=0.049) as their progenies at birth were heavier and larger respectively compared to offspring from nutrient restricted fed heifers. However, as calves aged, these differences disappeared (P>0.1). In contrast, NR80 calves had the highest (P=0.03) starter intake compared to control and NR120 calves. This difference persisted when rumination and feeding time was evaluated. However, no difference (P>0.1) in apparent total tract digestibility was observed despite the difference in starter intake. Results from this study indicate that heifer nutrition during early pregnancy can influence starter intake, birth weight and heart girth of progenies, and that altering maternal nutrition during early gestation can reprogram progenies behavioural performances. This emphasizes how crucial it is to manage the heifers' nutritional needs during the early stages of gestation. Investigation into the long-term effects of altering maternal nutrition during early gestation is recommended.

Key words: Energy restriction, pre-weaning, digestibility, starter intake, early-gestation, calves

4.0. INTRODUCTION

Evidence suggests that maternal plane of nutrition during pregnancy may influence overall *in utero* development of calves, with possible long-term effects on performance, longevity, fertility, and postpartum well-being (Mossa et al., 2013; Caton et al., 2019). This phenomenon has long been referred to as fetal programming or the baker hypothesis (Baker, 1992), although the biological mechanisms linking maternal nutrition during gestation to future offspring performance are not well understood. However, there are numerous instances in biology of nutritional interventions early in development, changing morphological and metabolic development in a way that lasts for a long time (Noya et al., 2019; Vautier and Cadaret, 2022).

All cells require energy to function, so physiologically, energy is essential for tissue maintenance, growth, milk synthesis, and embryonic development (Ghosh et al., 2023). The amount of energy needed for body maintenance depends on activity level and environmental stress (Most et al., 2019). According to ARC (1980) and INRA (2018), energy intake is the most significant factor influencing the growth rate and reproductive performance of cattle, so it is essential to accurately estimate the amount of energy needed for modern genotypes under modern feeding conditions to increase profitability and lessen the industry's negative environmental impact.

When energy is the limiting factor in the diet, rumen fill limits the intake (Tedeschi and Fox, 2020). However, pregnant ruminant animals fed limited energy diets may compromise their own body and tissue development and impair fetal growth and development, as such, influence the performance trajectory of their offspring reported in several studies (Moriel et al., 2016; Ramírez et al., 2020).

Generally, prenatal nutrition affects calves' weight at birth as there is compelling evidence from a recent meta-analysis study that investigated the associative effect of energy and protein intake during gestation (Barcelos et al., 2022). In the same study, the maternal energy status during late gestation was a predictor of the weaning weight of the calves. However, the bulk of the studies on prenatal energy intake on calves' overall postnatal performances have focused on periods between mid- and late gestation even though the early trimesters of pregnancy are known to be the critical stages for embryo and organ development (Reynolds and Redmer, 1995; Duarte et al., 2013; Gionbelli et al., 2017).

Nutrient restriction during pregnancy has been widely used to model offspring performance in post-natal life (Reynolds et al., 2021). Progenies from mothers that were subjected to nutrient restrictions during gestation have been shown to cause increased total fetal GIT weight (Meyer et al., 2010), altered digestive capacity in offspring (Noya et al., 2019) in calves or reduced enzymatic activities in lambs (Trotta et al., 2020). However, bovine models including overfeeding during gestation have equally shown inconsistent results in literature indicating that perturbation in maternal nutrition during gestation is a two-fold approach. For instance, CP and ME intake, at levels higher than those recommended by the NRC, pregnant cows in the third trimester of pregnancy reduced the progeny weight up to 205 days of age (Zago et al., 2020) while Chen et al. (2022) showed that beef cows fed high energy dense diets (NEm= 1.67 Mcal/kg of DM), 45 days to parturition produced calves with decreased growth, immunity and antioxidative capabilities than their counterparts.

Studying the effects of maternal nutrition on offspring overall performance have shown to depend on the timing of nutritional intervention (nutritional stress x time dependent manner), as tissue development occurs at specific times during gestation (McCoski et al., 2021). Besides the phenotypic expressions of progenies, several authors have found that maternal energy restriction during mid-late gestation results in offspring with greater temperament score (Gardner, 2017), greater insulin response (Radunz et al., 2012), reduced collagen content in *longissimus lumborum* (Mohrhauser et al., 2015), and lower titers after bovine diarrhea virus vaccination at 306 d of life (Moriel et al., 2016). Others have reported that nutrient restricted fed cows in early gestation produced offspring with underdeveloped gastrointestinal tract evidenced from poor milk replacer digestibility on day 25 post calving and lower IGF concentrations (Noya et al., 2019). However, the combined effects of nutrient restriction, timing, and duration of restriction on fetal organ growth and tissue development are less well known (Barcelos et al., 2022). This is probably due to the gestational difficulties of assessing fetal development at each stage of gestation without slaughtering the gravid animals.

Many previous studies have considered factors affecting nutrient digestion and behavior in calves mainly during pre and post weaning phases. Calves fed high milk replacers reduced solid feed intake and consequently reduced apparent total tract digestibility of nutrients (van Niekerk et al., 2020). In the same study, calves fed low amounts of milk replacers showed increased fibrolytic bacteria proportions at week 5 postnatal in fecal and rumen samples, which resulted in greater postweaning BW gain and similar BW and frame measurements by 16 weeks of age. Since fetuses receive all their nourishment from their mothers during pregnancy and their own GI tract and tissue development is taking place, the focus is now shifting to the time when the individual was a two celled embryo.

From the standpoint of fetal programming, male offspring who were born to mothers supplemented with 10% CP (control) compared to 5% CP (restricted group) in mid-gestation showed reduced digestibility of significant diet components between 382 and 445 days of age

(Nascimento et al., 2022). At 100 days, the progenies in the control group spent 52% more time per day eating supplements than their peers, and at d 210, they spent 17% less time being inactive. In the feedlot period, the males from the control group also ruminated for 15 minutes longer each day than their counterparts (Nascimento et al., 2022).

There is far less information available on whether there are postnatal feeding strategies that can achieve positive production, intake, health, and welfare traits in offspring born to mothers exposed to nutrient perturbations during embryonic and fetal development. Evidence in mice showed that total energy restriction during early pregnancy followed by adequate nutrition for the remainder of gestation, is particularly linked to cardiovascular and metabolic disease risks during adulthood (Van Gronigen Case et al., 2021). Piglets from mothers who were nutritionally deficient had longer small intestines than their peers, indicating that increased small intestinal absorption area may be a mechanism by which growth-restricted neonates increase their growth rate once they are in an external environment with unlimited access to food (Cao et al., 2014). Thus, fetal programming of milk producers could be explored to improve future offspring performance including growth, intake, and behavior (Moriel et al., 2021).

In general, replacement heifer- calf management programs are crucial because, in addition to being costly and affecting whether heifer- calves reach a 24-month age at first calving (AFC), the conditions that calves are exposed to from birth to weaning determine how much future investment they will make in the herd (Palczynski et al., 2020).

This study tested the hypothesis that calves born to heifers exposed to limited energy intake in early gestation would be characterized by low starter intake, poor behavioural patterns, and less total tract apparent digestibility due to inadequate formation of the GIT in utero. Therefore, this study sought to investigate the effects of feeding replacement dairy heifers a limited amount of their maintenance energy requirements in early gestation on feed intake, performance, behavior, and apparent total tract digestibility in their offspring during their pre-weaning life.

4.1. MATERIALS AND METHODS

All experimental animals were handled in accordance with DPR 27/1/1992 (Animal Protection Regulations of Italy) in conformity with European Community regulation 86/609 and were approved by the local Committee for the Animal Welfare of the University of Sassari, Italy (Prot.n0001848 of 2/5/2019). This study was conducted between January and May 2022 at the same commercial farm their mothers were housed.

4.1.1. Dam management during pregnancy

The experimental design during the maternal phase has been previously described in detail (Chapter 3). Briefly, based on body weight and age, Holstein Friesian heifers (n=28; BW±SD; 371.57±42.14, age±SD; 15.7±1.08 months) were divided into 3 groups, and from 11 days before conception were fed as following: (i) 0.6 M of their maintenance energy requirements (M) until day 80 of gestation (nutrient restricted NR80, n = 11), (ii) 0.6 M until day 120 of gestation (NR120, n=11), and (iii) ad libitum (1.8 M) until day 120 of gestation (control, n=6).

Heifers were group fed once the individual feeding period ended, with ad libitum access to feed until calving. Body weights and other linear measurements of heifers were measured once monthly while behavioural observations were conducted once in each trimester of pregnancy. Feed intake after the individual feeding regimen was predicted using established equations in literature. Experimental protocol, data analysis and results of heifers used in this study have been previously reported (Chapter 3).

4.1.2. Calves' management

Twenty-two single female calves (NR80; n=8, NR120; n=9, Control; n=5) were retained after calving, housed in single boxes, and managed under similar conditions until weaning. Four calves were males and were excluded from the trial. One calf from the NR120 group died from omphalophlebitis while another calf from NR80 presented abnormal ovarian follicles and were excluded from all calf analysis. Calves were fed colostrum within 6 hours of birth and for the first 4 consecutive days of post-natal life. From day 4 post-natal, starter and water were provided in addition to milk replacer (MR) where MR was fed twice a day (6 L/d) at 08:00 and 18:00 through a nipple bucket. Calves were monitored daily, and health status was assessed by trained study personnel and treated as needed by a veterinary reproduction and Medicine veterinarian.

Calves in the same block received an equal colostrum supply by pooling colostrum from different dams and preparing colostrum doses that were equal in quality or supplemented with artificial colostrum (≥22% Brix). If the calves were born when fresh good quality colostrum was available (i.e., during milking time of a fresh cows in the herd), they received fresh colostrum, whereas calves born away from milking time (i.e., at night) were fed previously frozen colostrum; colostrum was slowly thawed by inserting the hermetic container in water at high temperatures, until reaching 37°C, the optimal administration temperature. Furthermore, in the first two days, the colostrum was always supplemented with 2g of freeze-dried colostrum (Bayern Genetik^{TM}) in both daily meals.

4.1.3. Calves' health management

Around 10 days old post-natal, some cases of neonatal diarrhea occurred (*Cryptosporidium spp.)* and were diagnosed with rapid antigenic tests (Kerbl). All calves were treated with a Paromomycin-based drug (Parofor crypto^{TM}, Huvepharma; 2 ml/10 kg of body weight for 7 days). The same drug was administered for preventive purposes in the calves born late, starting from day 4 of life. Furthermore, when some calves where one month old, cough and nasal discharge were visible, in some cases accompanied by fever. The calves were carefully examined, nasal swabs were collected and *Pasteurella* infection was diagnosed. The calves with fever and nasal discharge at the visit were administered antibiotic treatment (Alamycin LA 300^{TM}, oxytetracycline dihydrate 1 ml/kg every three days for one week). In addition, a thoracic ultrasound was performed to monitor the health of the calves.

4.2. Linear body and feed intake measurements

Body weight and linear body measurements (thoracic circumference, hip height and height at withers) of calves were assessed immediately after birth and every fourteen days until weaning (age±SD; 69±7 days). All body measurements were taken before morning milk feeding. The body condition scores (BCS) were taken by two trained evaluators using a scale from 1 to 5 points (in which 1 = emaciated and 5 = obese calves). The final score was defined as the average values obtained from each evaluator. Calves received the same diet until weaning. However, starting 10 days to the end of weaning, calves were introduced to a gradual weaning process by limiting the quantity of MR by 0.5 L/d (Step down weaning; Khan et al., 2007b). All calves were weaned individually and at d 74 of postnatal life in accordance with the weaning procedure of the commercial farm.

4.3. Feeding and Chemical analysis of feed

Starter feed was obtained from a commercial mix supplied by Purina®. Starter intake was recorded every 3 days and adjusted as the calves aged. Feed and orts were sampled monthly and analysed for chemical composition (Table 4.1). Water was introduced from day 3 of postnatal life. Feed samples were analyzed for DM (105°C for 24 h), CP, ether extract, and ash (AOAC International, 2000; methods 988.05, 920.39, and 942.05 respectively), NDF (using heat-stable α-amylase; Mertens, 2002), ADF (AOAC, 1990; method 973.18), and ADL (Robertson and Van Soest, 1981). Non-Fiber carbohydrates (% of DM) were calculated as 100 − (NDF + CP + ash + ether extract). Detailed feed analysis has been reported in the preceding Chapter.

4.4. Behavioural observations

To investigate behavioural outcomes in the early preweaning period, selected female calves from each group were monitored during a 12-hour diurnal direct live observation at postnatal weeks 3 and 4. The trained observer was positioned at least 15 m from the calves' boxes to ensure unobstructed views and avoid animal contact. Animals were observed at 3-min intervals as described by DeVries et al. (2003) during the following activities: Feeding, lying, rumination, and other activities (walking, drinking, or non-recumbent activities). Detailed activities of behavioural patterns have been described in Chapter 3. A total of 8640 behavioural observations were recorded during the pre-weaning period.

4.5. Apparent Total-Tract Nutrient Digestibility

Fecal measurements were performed according to the procedure described by Luan et al. (2016). Fecal samples were collected from the rectum of each calf into plastic containers at 6 time points (TP) for 3 consecutive days: TP1, d 1, 0800 h; TP2, d 1, 1600 h; TP3, d 2, 0900 h;

TP4, d 2, 17:30 h; TP5, d 3, 1200 h; TP6, d 3, 1900 h and the samples were directly stored at –20°C until analyzed. Fecal samples from TP1 to TP6 were analyzed for DM, CP, NDF, starch and ADL contents. Starter feed and orts intake were recorded daily and sampled to estimate apparent total tract digestibility. Acid detergent lignin was used as a marker to calculate the apparent total-tract digestibility (Kotb and Luckey 1972) according to the following equation described in Chapter 3.

4.6. Data analysis

Calves' variables (BW, ADG, intake) were analysed as a mixed model with repeated measures in R (R Core Team, 2020).

$$Y_{ijk} = \mu + \text{Treatment}_i + \text{Time}_j + (\text{Treatment*Time})_{ij} + \text{ID}_k + \varepsilon_{ijk}$$

The model included the fixed effects of feeding program during pregnancy (Treatment), time (T), and treatments × time (T), and calves as random effects. For behavioural and apparent total tract digestibility, data were analysed using One-way ANOVA. We declared all dependent variables significant when $P \leq 0.05$ and a tendency to differ when $P > 0.05 < 0.10$. When treatment means differed, a post hoc TukeyHSD test was performed.

4.7. RESULTS

4.7.1 Starter intake and body measurements

There was a treatment effect in voluntary dry matter starter intake over the entire preweaning phase (P=0.003). Calves born to NR80 group had higher starter intake (n = 8;1528±66.9g) than those from NR120 (n = 9;1392.7±54.4g, P=0.04) and Control (n = 5;1328.1±77.2g, P=0.006), respectively, while calves from control and NR120 fed heifers had similar starter DMI (P=0.565). However, no interactive effect (P=0.940) in starter intake was observed in progenies throughout their preweaning phase (Figure 4.1).

The body weights (Figure 4.2) of calves at birth were significantly different (P = 0.017); however, remained similar at weaning (P=0.698). Control fed heifers produced heavier calves at birth compared to NR80 fed heifers, while daughter calves from Control fed heifers tended to be heavier than NR120 calves. In addition, no interaction effect (P=0.302) in body weight between the experimental groups and calves age was detected during the pre-weaning period. Heart girth, expressed in cm, of progenies from control fed heifers was larger than their counterparts (P=0.049). Hip height tended to differ (P=0.069) while wither height (expressed in cm) at birth across all treatment groups was similar (P=0.677). Unlike in body weight, heart girth and hip height, there tended to be a difference (P=0.102) in wither heights (Table 4.2) as calves aged. Although BCS was not measured at birth, no statistical difference (P=0.515) nor an interactive effect (P=0.191) in BCS across the different groups was detected. Interestingly, the combined effects on weight gain or loss in heifers during early gestation were not associated with the birth body weight of calves from nutrient restricted mothers (r = 0.05, P=0.789).

As expected, a strong position correlation was observed between body weight and heart girth (r = 0.96), hip height (r = 0.90), wither height (r = 0.92) and back length (r = 0.82). Result on average daily gain is presented in Figure 4.3. Effect of maternal energy diet during early gestation on average daily gain (ADG) from birth to weaning tended to differ (P=0.08) among calves from nutrient restricted fed mothers (500±0.1g/d, 600±0.04g/d, and 600±0.04g/d: Control, NR80, and NR120, respectively). No interaction effect was detected between treatment and time for ADG (P=0.985). In addition, no association was found between ADG of nutrient restricted mothers in the first 80 days of gestation and ADG of their progenies during the preweaning period (r = -0.08, P=0.469).

Similarly, no association was found between ADG in control fed heifers during the first 120 d of gestation and their progenies (r=0.25, P=0.154). In addition, there was no associative effect of the body weight gain in pregnant heifers in late gestation on overall ADG of their progenies (r =-0.05, P=0.619).

4.7.2 Calves behavioural pattern

Calves born to NR80 dams spent more time visiting the feed bunk and eating than their peers at 3 and 4 weeks of age (P<0.05). However, this did not translate into more time ruminating as NR120 calves spent more (P=0.004) time ruminating (154 min/12 h) than calves in NR80 (134 min/12 h) and Control (82 min/12 h). Heifer calves in the control group spent the least amount of time eating and ruminating (Table 4.3). Time spent lying was higher in Control calves and least in NR120 calves (P<0.0001).

Calves born to mothers fed 0.6 M of their maintenance energy requirements during the first 120 d of gestation spent the most time carrying out other activities compared to those from NR80 and the Control (P=0.01). Indeed, the behavioural pattern presented by these calves shows maternal energy restriction in early gestation influences behavioural patterns in female offspring in early pre-weaning stage.

4.7.3 Apparent total tract digestibility

No difference (P>0.1) in dry matter, crude protein, starch and NDF apparent total tract digestibility was observed (Table 4.4).

4.8. DISCUSSION

This study tested the hypothesis that maternal energy restriction during early gestation and day of gestation in dairy heifers would affect the birth weight, behavioural patterns, starter intake, growth trajectory and apparent total tract digestibility of their offspring during the preweaning period. Furthermore, it was speculated that heavier mothers during pregnancy would produce heavier calves at birth. In this study, a difference in the birth weight and heart girth of calves across the treatment groups was detected. Other linear body measurements of calves born to mothers exposed to either nutrient restriction or overfeeding in early gestation did not differ at birth, however, a tendency to differ in height at withers was detected during preweaning. Immediate short-term effects of altering maternal energy requirements from preconception to early gestation showed that behavioural patterns can be reprogramed in calves evidenced from differences in rumination and feeding times. Irrespective of experimental groups, total starter intake was similar among calves with calves consuming more than 1100g/d of starter prior to weaning. However, with NR80 calves consuming more starter than their counterparts, this difference was not evident in total tract apparent nutrient digestibility as all calves showed similar outcomes.

There is conflicting evidence in the literature regarding how early-gestation maternal dietary restriction affects dairy calf health and growth. In general, most studies found that adequate nutrition in late pregnancy can relieve the effects of feed restriction during early pregnancy on offspring growth with minor effects on postnatal growth (Keyon and Blair, 2014; Bell and Greenwood, 2016). This may help to explain why the progenies' phenotypic expressions throughout the preweaning stage of the current investigation showed no differences from one another. Additionally, the presence of a difference in birth weight in the current study is inconsistent with earlier findings from our group (Mossa et al., 2013). However, several previous studies have reported that alterations in maternal nutrition during pregnancy might affect fetal growth and birth weight in cattle (Caton et al., 2009; Neville et al., 2010).

Because the evidence is compelling that the phenotype of some animals is compromised by events happening before, they are born, our results on body weight at birth is consistent with previous reports by Ford and colleagues (2007) who demonstrated that lambs born to nutrient restricted (50% of their nutrient requirements) ewes during early gestation had decreased in birth weights. However, it contrasts the conclusion drawn by the authors (Ford et al., 2007) who reported a significant catch-up growth as the lambs weighed more at 4 months postnatal and at slaughter than their counterparts. This was not observed in the current study. In another study, lambs born from late-gestation maternal energy restricted mothers had similar weights at birth compared to their contemporaries but differed in thoracic circumference (McGovern et al., 2015).

These studies highlighted above to some extent support the speculations that more severe impact of altering maternal nutrition during gestation reprograms body weight at birth. However, pronounced effect over the entire preweaning period is evident when nutrition is altered in late gestation where detailed nutritional management is historically considered (McGovern et al., 2015; Lopes et al., 2020; Abuelo, 2020). Based on our results, we partially accept our initial hypothesis since control fed heifers produced heavier calves at birth; however, this superiority disappeared during the pre-weaning period.

Nevertheless, in the current study, when the period between birth and 30 days post-calving was considered, a tendency for body weights to differ was observed with higher weights gained to control calves. As previously mentioned, there is no clear-cut response to maternal stressors during pregnancy on offspring performance. The combined effects of the timing, duration, and level of stress on pregnant dams on their progenies is still inconclusive.

Furthermore, the current study partially rejects the theory that offspring from nutrient restricted fed heifers would continue to adapt to a less limited nutrient from fetal environment throughout life (Vautier and Cadaret, 2022), where they show an exponential catch up growth and possibly outweigh their counterparts during pre-weaning. This could be because the nutrient restriction applied in our study shortly before conception and during early gestation was not sufficient to induce major changes in fetal skeletal frame during pregnancy that could persist until weaning. Another assumption may be an increased plasma leptin concentrations which favours fat deposition over muscle growth (Ford et al., 2007). However, although leptin was not measured in this study, more studies are needed to assess the long-term consequences of a maternal weight loss before refeeding on progenies performance throughout pre-weaning.

A recent review by Wathes (2022) highlighted the need for careful evaluation of catch-up growth in sheep and beef since prenatal nutrition could also affect the dam's colostrum quality and milk production capacity. Since individual milk production capacity was not measured or that calves did not received colostrum directly from their mothers in the current study, more studies are needed to investigate this hypothesis.

Despite the tendency to differ in ADG across treatment groups in the current study, calves did not maintain a growth rate of >750g/d that is required to achieve a body weight and stature to calve at 24 months (Wathes et al., 2014). Although, the increased maternal ADG during pregnancy was not associated with those of the calves, the increased calves' growth rates from day 44 onwards can be explained in parts; either in reference to the energy to protein ratio in the milk replacer was adequate as evidence suggest that under thermoneutral conditions, a 45 kg calf requires about 325 g/day of whole milk solids (2.5 L/day) or 380 g/day of calf milk replacer (CMR), which comprise about 22.5 MJ ME/kg and 19.5 MJ ME/kg, respectively, of ME and excess nutrients to promote growth (Drackley, 2008). This reported energy level is similar in composition with the milk replacer, containing19.8 MJ ME/kg, fed in the current study. Nonetheless, there are studies that have shown that *in utero* heat stressed calves had lower weaning weight and ADG during preweaning (Monteiro et al., 2016a; Dado-Senn et al., 2020b) regardless of milk replacers or starter feed administered.

According to Dwyer et al. (2003) and Paranhos da Costa et al. (2008), neonatal behavior plays a vital role in the survival of the animal in precocious species like calves. For example, Alexander and Peterson (1961) reported that up to 30% of lamb deaths are a direct result of the lamb's own poor behavior. In the present study, there is evidence that behavioural patterns in calves can be reprogrammed. For instance, progenies from whose mothers were exposed to early- gestation nutrient restriction had higher rumination time than their counterpart. This is mostly likely the marked increase in rumination time in the second and third trimester of pregnancy in their mothers who had recorded rumination times below the minimum threshold of >360mins/day for adult cows during early gestation.

It could be speculated that nutrient restriction in early gestation improves rumination activities in later stages of pregnancy in dams and positively influences rumination time in their daughters during early post-natal life. This needs to be further investigated. However, the amount of minutes calves spent ruminating, resting, and feeding was below reported values for calves of similar age and weight (Swanson and Harris, 1958; Burfeind et al., 2011; Neja, 2013). Interestingly, up until the calf is around 4 to 6 weeks old, the rumination times increase before levelling off at about 5 hours per day (Margerison et al., 2003). Nonetheless, the start of rumination at post-natal week 3 in the current study indicates the continued development of the rumen (Wang et al., 2022).

Information on colostrum, milk replacer and starter intake in pre-weaned dairy calves is important indicator of performance and welfare of the animals. To properly support rumen development, the dairy calf will often be given an additional concentrate starter feed, either with or without grass. When they are ingesting 1 to 1.5 kg of concentrate feed in the days leading up to weaning, they are deemed to be ready for weaning (AHDB, 2020). However, research indicates that calves' intakes will differ substantially (Webb et al., 2014). For instance, calf concentrate intakes were as low as 500 g/d (Leal et al., 2021), despite a step-down milk weaning schedule in the weeks before expected weaning. This may be a consequence of high rates of milk feeding and /or because sub-optimal supplementary feeds are offered.

Calves in the present study consumed more than 1 kg of starter per day during the transition weaning phase, which is consistent with values reported by (AHDB, 2020). Indeed, individual calf intake varied with NR80 calves consuming more starter than their counterparts in the present study. In contrast, while lambs from mothers restricted to 80% ME in late gestation had lesser colostrum intake than over-nourished mothers (McGovern et al., 2015), no difference was detected in starter intake in calves born to either mothers with low or high BCS during late gestation (Alharthi et al., 2021).

Offering a total mixed ration (TMR) could be used as an alternative to starter feed. TMRs are frequently offered to adult dairy cows to improve intakes and provide a more stable rumen environment (Schingoethe, 2017). When compared to concentrate mixed with ground hay, TMR made from concentrates and longer cut hay increased body weight and rumination durations in young calves (Montoro et al., 2013). This reported alternative feeding technique may be employed to satisfy milk-fed dairy calves' appetite (Palczynski et al., 2020).

Despite the differences in starter intake in daughters from different experimental groups, apparent total tract digestibility was similar among calves. Generally, higher starter DMI is associated with low nutrient digestibility due to high passage rate (Seo et al., 2006). This contrasts the current findings, as NR80 calves, with higher starter intake, showed similar DM digestibility compared to their peers. It is unclear why nutrient digestibility was comparable among calves even when digestibility components were investigated in the last three days of weaning, coinciding with drastic milk reduction and an increase in starter intake by calves. One theory could be that since calves are typically housed on hay beddings, estimates of digestibility are sometimes tainted by potential consumption of hay; however, this is not a well-established theory (Hill et al., 2019). Another potential reason maybe the increased capacity of the gut associated with increased DMI despite high passage rate (Broesder et al., 1990).

4.9. CONCLUSION

Body weight and stature remained similar throughout preweaning, suggesting that daughter calves, regardless of their mother's nutritional status in early gestation, had a similar potential to grow and achieve equal weight gain. However, altering the level of maintenance energy requirements offered to replacement dairy heifers in early gestation had a significant influence on calves' weight and thoracic circumference at birth, and overall solid feed intake during pre-weaning. Although calves from control fed heifers were heavier at birth and tended to be taller than calves from nutrient restricted heifers, these differences did not translate into higher starer intake nor rumination time. Contrary to our hypothesis, apparent total tract digestibility was similar among all calves. Collectively, the findings from the current study suggests that dairy heifers subjected to a short energy restriction in early gestation can reprogram offspring performance with better starter intake performance and behavioural patterns in the pre-weaning period, further suggesting that it could be an adaptive mechanism of these calves born lighter to meet up with the requirement for their growth and survival. Further investigation of the long-term effects of maternal nutrient restriction on overall calf performance is recommended.

Table 4.1. Chemical composition of starter diet (% DM basis)

Item	Composition
DM	89.6
CP	19.3
NDF	34.2
ADF	23.9
ADL	4.8
EE	2.6
Ash	7.7
ADIP	1.7
Sugars	12.8
SOLP	6.5
NDIP	5.8
NDF24	49.1
Starch	9.3
[1]NFC	36.2
[2]ME (Mcal/kg)	2.65

[1] Calculated as NFC = 100 − CP − ash − NDF − ether extract.

[2] Calculated using NRC, 2001 guidelines.

NDIP= neutral detergent insoluble protein, ADIP= acid detergent insoluble protein, SOLP= soluble protein. Commercial starter feed composed of Wheat chop, beet pulp gluten wheat flour, corn cob, gluten corn bran, sunflower seed extracted flour, soybean husks, wheat bran, sugar beet molasses, maize germ expeller, maize from genetically modified corn, dehulled soybean meal, sunflower expeller, inactivated yeast product, rice husk, wheat distillates. Sugars = 12.8%, Ca = 0.7%, P = 0.4%, Mg = 0.2%, K = 1.6%.

Table 4.2. Body dimension in pre-weaned calves (Mean±SE) born to early gestation maternal energy nutrient restricted or overfed heifers

Item	Control (n = 5)	NR120 (n = 9)	NR80 (n = 8)	p-value Treat	 Age	 Treat*Age
Heart girth cm	85.1±1.5	83.5±1.1	84.4±1.3	0.205	<0.0001	0.372
Hip height cm	84.3±1.1	82.7±0.8	82.1±0.9	0.138	<0.0001	0.325
Wither height cm	80.1±0.9	78.8±0.8	78.5±0.9	0.186	<0.0001	0.102
Back length cm	64.8±1.3	65.5±0.8	64.8±1.1	0.772	<0.0001	0.502
BCS	2.8	2.8	2.9	0.824	<0.0001	0.191
[1]Gain:feed	2.2±0.2	2.2±0.1	2.2±0.1	0.919		
Body weight, kg	56.1±2.6	54.8±2.1	55.1±2.4	0.515	<0.0001	0.302

Treat: Treatment during pregnancy, BCS: Body condition score

[1] Gain to feed ratio was calculated as total weight gain during the preweaning divided by total feed intake for the same period. NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

Table 4.3. Twelve hours diurnal behavioural patterns in pre-weaned calves born to early gestation maternal energy nutrient restricted or overfed mothers

Item (Mins/12h)	Treatment			p-value	SEM
	Control (n=4)	NR80 (n=6)	NR120 (n=6)		
Feeding	39^{b}	47^{a}	35^{ab}	0.021	0.002
Rumination	82^{c}	134^{b}	154^{a}	<0.0001	0.004
Resting	360^{a}	301^{b}	268^{c}	<0.0001	0.005
Others	239^{b}	238^{bc}	262^{a}	0.010	0.005

a–c Means within a column with different superscripts differ ($p \leq 0.05$), Others = combination of activities including drinking, vocalization, walking and standing without eating. Calves were born to mothers either fed; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

Table 4.4 Apparent total tract digestibility of pre-weaned female calves (Mean±SE) born to early gestation maternal energy nutrient restricted or overfed mothers

Item	Treatment			p-value
(%)	Control (n=5)	NR120 (n=8)	NR80 (n=7)	
DM	73.2±2.1	72.3±1.3	72.1±1	0.8674
CP	82±1.7	80.9±0.8	80.3±0.7	0.558
NDF	48.9±2.9	49.7±1.4	49.7±1.3	0.947
Starch	92.3±0.9	92±0.8	92.2±0.9	0.966

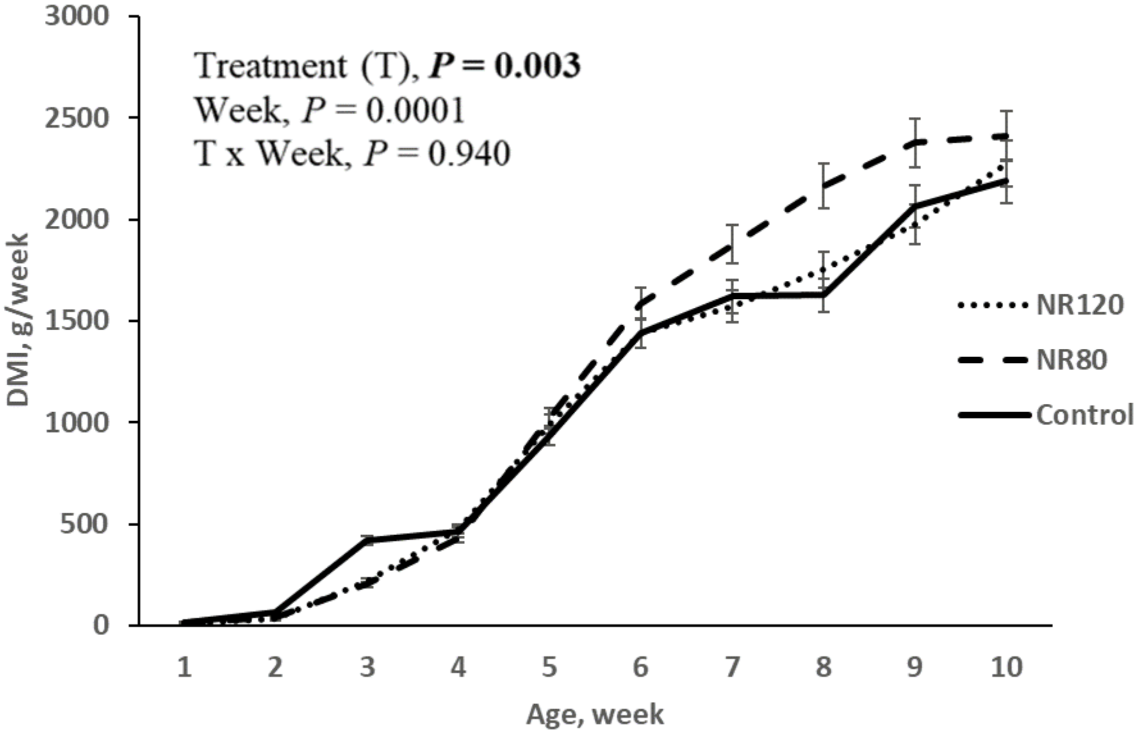


Figure 4.1. Voluntary starter DMI of female calves (n =22) during pre-weaning. Calves were born to early-gestation nutrient restricted versus overfed heifers. Calves were born from mother fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean.

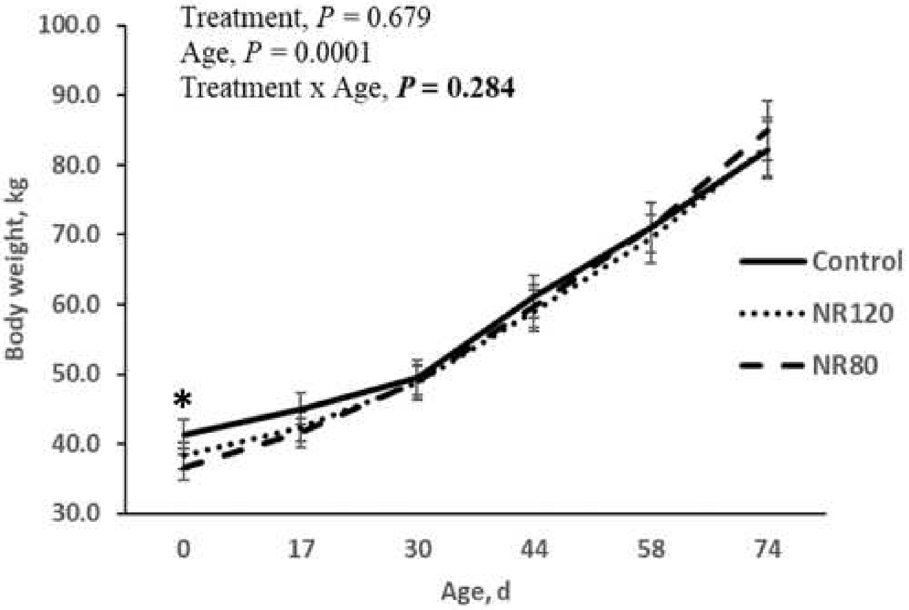


Figure 4.2. Body weight of female calves (n =22) born to early-gestation nutrient restricted versus overfed heifers. Largest SEM = 2.58. * = P<0.05. Calves were born from mother fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean.

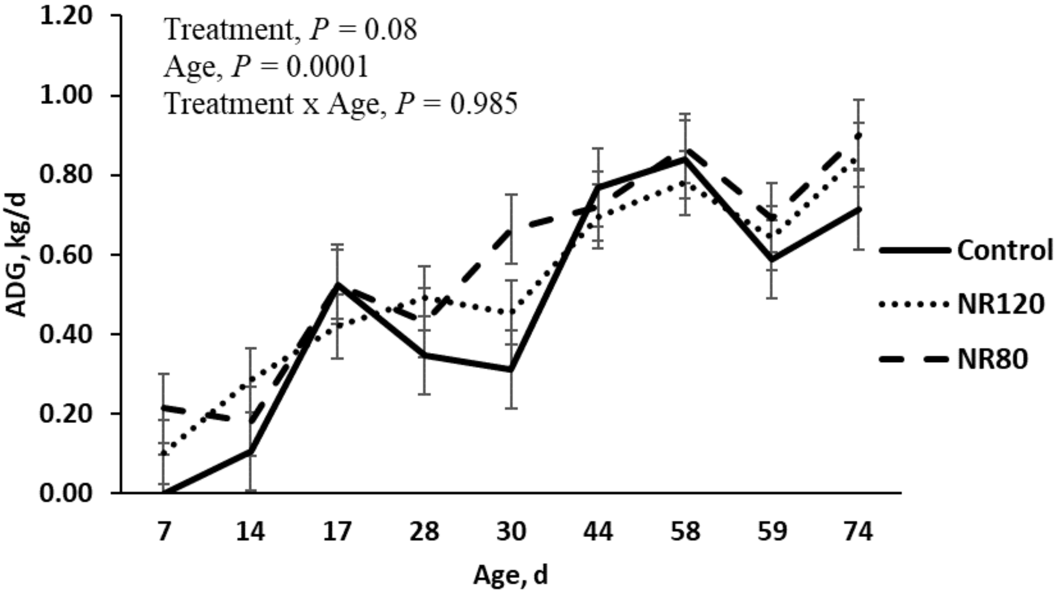


Figure 4.3. Average daily gains (ADG) of female calves (n =22) born to early-gestation nutrient restricted versus overfed heifers. Largest SEM = 0.1. Calves were born from mother fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. Error bars represent standard error of mean. Calves gradually transitioned from milk replacer to complete solid feeding from day 59 of age.

REFERENCES

Agricultural Research Council (ARC) 1980. The Nutrient Requirements of Ruminant Livestock; Agricultural Research Council, and The Gresham Press: London, UK, 1980.

AHDB (Agriculture and Horticulture Development Board). 2017. Better Calf Housing.

Alexander, G. and Peterson, J. 1961. Neonatal mortality in lambs. Australian Veterinary Journal 37:371–381. doi:10.1111/j.1751-0813.1961.tb03789.x.

Alharthi, A.S., Coleman, D.N., Alhidary, I.A., Abdelrahman M.M., Trevisi, E. and Loor, J.J. 2021. Maternal body condition during late pregnancy is associated with in utero development and neonatal growth of Holstein calves. Journal of Animal Science and Biotechnology. 12: 44. https://doi.org/10.1186/s40104-021-00566-2.

Barcelos, S.d.S., Nascimento, K.B., Silva, T.E.d., Mezzomo, R., Alves, K.S., de Souza Duarte, M. and Gionbelli, M.P. 2022. The Effects of Prenatal Diet on Calf Performance and Perspectives for Fetal Programming Studies: A Meta-Analytical Investigation. Animals, 12, 2145. https:// doi.org/10.3390/ani1216214.

Bell A.W., Greenwood P.L. 2016. Prenatal origins of postnatal variation in growth, development, and productivity of ruminants. Animal Production Science; 56:1217–1232. doi: 10.1071/AN15408.

Boulton, A.C., Rushton, J. and Wathes, D.C. 2017. An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. Animal, *11*, 1372–1380.

Broesder, J.T., Judkins, M.D., Krysl, L.J., Gunter, A. and Barton, R.K. 1990. Thirty or sixty percent milk replacer reduction for calves; effects on alfalfa hay intake and digestibility, digestive kinetics and ruminal fermentation. Journal of Animal Science. 68:2971-2985.

Burfeind, O., Schirmann, K., von Keyserlingk, M.A.G., Veira, D.M., Weary, D.M. and W. Heuwieser, W. 2011. Technical note: Evaluation of a system for monitoring rumination in heifers and calves Journal of Dairy Science. 94:426–430 doi:10.3168/jds.2010-3239.

Cao, M.M.A., Lianqiang, C., Wang, J.M.A., Yang, M.M.A., Guoqi-Su, M.A., Zhengfeng, F., Yan, L., Shengyu, X. and Wu, D. 2014. Effects of maternal over- and undernutrition on intestinal morphology, enzyme activity, and gene expression of nutrient transporters in newborn and weaned pigs. Nutrition, 30(2014): pp. 1442–1447. https://doi.org/10.1016/j.nut.2014.04.016

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

Caton, J.S., Crouse, M.S., Reynolds, L.P., Neville, T.L., Dahlen, C.R., Ward, A.K. and Swanson, K.C. 2019. Maternal nutrition and programming of offspring energy requirements. Translational Animal Science*.*, 3(3):976–990.

Chen, H., Wang, C., Huasai, S. and Chen, A. 2022. Effect of prepartum dietary energy density on beef cow energy metabolites, and birth weight and antioxidative capabilities of neonatal calves. Scientific Reports, 12:4828. https://doi.org/10.1038/s41598-022-08809-6.

Dado-Senn, B., Quellet, V., Dahl, G.E. and Laporta, J. 2020b. methods for assessing heat stress in pre-weaned dairy calves exposed to chronic heat stress or continuous cooling. Journal of Dairy Science. 103:8587-8600.

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

Dwyer, C. M., Lawrence, A.B., Bishop, S.C. and Lewis, M. 2003. Ewe-lamb bonding behaviours at birth are affected by maternal undernutrition in pregnancy. British Journal of Nutrition. 89:123–136. doi:10.1079/BJN2002743.

Ford, S.P., Hess, B.W., Schwope, M.M., Nijland, M.J., Gilbert, J.S., Vonnahme, K.A. et al. 2007. Maternal Undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. Journal of Animal Science. 85:1285-1294. Doi:10.2527/jas.20045-624.

Gardner, Jose, "Effects of Gestational Dietary Intake on Calf Growth and Early Feedlot Performance of Offspring" (2017). MSc Thesis, University of Utah, USA https://digitalcommons.usu.edu/etd/5395.

Ghosh, S., Körte, A., Serafini, G., Yadav, V. and Rodenfels, J. 2023. Developmental energetics: Energy expenditure, budgets, and metabolism during anima embryogenesis. Seminars in Cell & Developmental Biology, 138: 83-93. https://doi.org/10.1016/j.semcdb.2022.03.009.

Gionbelli, T., Rotta, P., Veloso, C., Valadares Filho, S., Carvalho, B., Marcondes, M., Ferreira, M., Souza, J., Santos, J. and Lacerda, L. 2017. Intestinal development of bovine foetuses during gestation is affected by foetal sex and maternal nutrition. Journal of Animal Physiology and Animal Nutrition, 101:493–501.

Greenwood P.L., Thompson A.N., Ford S.P. 2009. Postnatal consequences of the maternal environment and of growth during prenatal life for productivity of ruminants. In: Greenwood P., Bell A., Vercoe P., Viljoen G., editors. Managing the Prenatal Environment to Enhance Livestock Productivity. Springer; Dordrecht, The Netherlands: 2009.

Hill, T. M., T. S. Dennis, F. X. Suarez-Mena, J. D. Quigley, K. M. Aragona, and R. L. Schlotterbeck. 2019. Effects of free-choice hay and straw bedding on digestion of nutrients in 7-week-old Holstein calves. Applied Animal Science. 35:312–317. https://doi.org/10.15232/aas .2019-01855.

Institut National de la Recherche Agronomique (INRA). INRA Feeding System for Ruminants; Wageningen Academic Publishers: Wageningen, The Netherlands, 2018.

Kenyon P.R., Blair H.T. 2014. Foetal programming in sheep—Effects on production. Small Ruminant Research; 118:16–30. doi: 10.1016/j.smallrumres.2013.12.021.

Lopes, R., Sampaio, C., Trece, A., Teixeira, P., Gionbelli, T., Santos, L., Costa, T., Duarte, M. and Gionbelli, M. 2020. Impacts of protein supplementation during late gestation of beef cows on maternal skeletal muscle and liver tissues metabolism. Animal, 14, 1867–1875.P3882-3893, APRIL 01, 2020

Margerison, J. K., Preston, T.R., Berry, N. and Phillips, C.J.C. 2003. Cross-sucking and other oral behaviours in calves, and their relation to cow suckling and food provision. Applied Animal Behavioral Science. 80:277–286.

McGovern, F.M., Campion, F.P., Sweeney, T., Fair, S., Lott, S. and Boland, T.M. 2015. Altering ewe nutrition in late gestation: II. The impact on fetal development and offspring performance. Journal of Animal Science, 93:4873–4882 doi:10.2527/jas2015-9020.

Mohrhauser, D.A., Taylor, A.R., Gonda, M.G., Underwood, R.R., Pritchard, R.H., Wertz-Lutz, A.E. and Blair, A.D. 2015. The influence of maternal energy status during mid-gestation on beef offspring tenderness, muscle characteristics, and gene expression. Meat Science, 110: 201-211. https://doi.org/10.1016/j.meatsci.2015.07.017.

Monteiro, A.P.A., Guo, J., Weng, X., Ahmed, B.M., Hayen, M.J., Dahl, G.E., Bernard, J.K. and Tao, S. 2016a. Effect of maternal heat stress during the dry period on growth and metabolism of calves. Journal of Dairy Science. 99(5): 3896-3907.

Montoro, C., Miller-Cushon, E.K., DeVries, J. and Bach, A. 2013. Effect of physical form of forage on performance, feeding behavior, and digestibility of Holstein calves. Journal of Dairy Science. 96:1117-1124.

Moriel, P., Piccolo, M.B., Artioli, L.F., Marques, R.S., Poore, M.H. and Cooke, R.F. 2016. Short-term energy restriction during late gestation of beef cows decreases postweaning calf humoral immune response to vaccination. Journal of Animal Science. 94(6):2542-52.

Mossa, F., Carter, F., Walsh, S.W., Kenny, D.A., Smith, G.W., Ireland, J.L., Hildebrandt, T.B., Lonergan, P., Ireland, J.J. and Evans, A.C. 2013. Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. Biology of Reproduction. 88:92.

Most, J., Dervis, S., Haman, F., Adamo, K.B. and Redman, L.M. 2019. Energy Intake Requirements in Pregnancy. Nutrients;11(8):1812. doi: 10.3390/nu11081812.

Nascimento, K.B., Galvão, M.C., Meneses, J.A.M., Moreira, G.M., Ramírez-Zamudio, G.D., Souza, S.P.d., Prezotto, L.D., Chalfun, L.H.L., Duarte, M.d.S., Casagrande, D.R. and Gionbelli, M.P. 2022. Effects of Maternal Protein Supplementation at Mid-Gestation of Cows on Intake, Digestibility, and Feeding Behavior of the Offspring. Animals 2022, 12, 2865. https:// doi.org/10.3390/ani12202865.

Neja, W. 2013. Behaviour of Calves in the first week of life. Journal of Central European Agriculture, 14(1): 33-41.

Neville, T. L., Caton, J.S., Hammer, C.J., Reed, J.J., Luther, J.S., Taylor, J.B., Redmer, D.A., Reynolds, L.P. and Vonnahme, K.A. 2010. Ovine offspring growth and diet digestibility are influenced by maternal selenium supplementation and nutritional intake during pregnancy despite a common postnatal diet. Journal of Animal Science. 88:3645–3656. doi:10.2527/jas.2009-2666.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Palczynski, L.J., Bleach, E.C.L., Brennan, M.L. and Robinson, P.A. 2020. Appropriate Dairy Calf Feeding from Birth to Weaning: "It's an Investment for the Future". Animals*,* 10, 116. https://doi.org/10.3390/ani10010116.

Paranhos da Costa, M.J.R.; Schmidek, A. and Toledo, L.M. 2008. Mother–offspring interactions in Zebu cattle. Reprod. Domest. Anim. 2008, 43, 213–216.

R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020.

Radunz, A.E., Fluharty, F.L., Relling, A.E., Felix, T.L., Shoup, L.M., Zerby, H.N. and Loerch, S.C. 2012. Prepartum dietary energy source fed to beef cows: II. Effects on progeny postnatal growth, glucose tolerance, and carcass composition. Journal of Animal Science, 90(13): 4962–4974, https://doi.org/10.2527/jas.2012-5098

Ramírez, M, Testa, L.M., López Valiente, S., Latorre, M.E., Long, N.M., Rodriguez, A.M., Pavan, E and Maresca, S. 2020. Maternal energy status during late gestation: Effects on growth performance, carcass characteristics and meat quality of steers progeny. Meat Science; 164:108095. doi: 10.1016/j.meatsci.2020.108095.

Reynolds, L., McLean, K.J., McCarthy, K.L., Diniz, W.J.S., Menezes, A.B., Forcherio, J.C., Scott, R.R. Borowicz, P.P., Ward, A.K., Dahlen, C.R. and Caton, J.S. 2021. Nutritional Regulation of Embryonic Survival, Growth, and Development. Advances in Experimental Medicine and Biology. https://doi.org/10.1007/978-3-030-85686-1_4

Schingoethe, D.J. 2017. A 100-Year Review: Total mixed ration feeding of dairy cows. Journal of Dairy Science. 100(12):10143-10150. doi: 10.3168/jds.2017-12967.

Seo, S., Tedeschi, L.O., Schwab, C.G., Garthwaite, B.D. and Fox, D.G. 2006. Evaluation of the passage rate equations in the 2001 dairy NRC model. Journal of Dairy Science. 89:2327-2342.

Swanson, E.W. and Harris, J.D. 1958. Development of Rumination in the Young Calf. Journal of Dairy Science, 41(12):1768-1776.

Tedeschi L.O. and Fox, D.G. 2020. Ruminant Nutrition System, Vol. I — An Applied Model for Predicting Nutrient Requirement and Feed Utilization in Ruminants. ISBN: 978-1-97507-701-3.

Trotta, R.J., Keomanivong, F.E., Peine, J.L., Caton, J.S. and Swanson, K.C. 2020. Influence of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activity of lamb offspring. Livestock Science. 241: pp. 104246. https://doi.org/10.1016/j.livsci.2020.104246

Van Gronigen Case, G., Storey, K.M., Parmeley, L.E. and. Schulz, L.C. 2021. Effects of maternal nutrient restriction during the periconceptional period on placental development in the mouse. PLoS ONE 16(2021): e0244971. https://doi.org/10.1371/journal.pone.0244971

van Niekerk, J.K., Fischer-Tlustos, A.J., Deikun, L.L., Quigley, J.D., Dennis, T.S., Suarez-Mena, F.X., Hill, T.M., Schlotterbeck, R.L., Guan, L.L. and Steele, M.A. 2020. Effect of amount of milk replacer fed and the processing of corn in starter on growth performance, nutrient digestibility, and rumen and fecal fibrolytic bacteria of dairy calves. Journal of Dairy Science, 103:2186–2199.

Wang, S., Diao, Q.Y., Hu, F.M., Bi, Y.L., Piao, M.Y., Jiang, L.S., Sun, F., Li, H. and Tu, Y. 2022. Development of ruminating behavior in Holstein calves between birth and 30 days of age. Journal of Dairy Science. 105(1): 572-584. https://doi.org/10.3168/jds.2021-20405.

Wathes, D.C. 2022. Developmental Programming of Fertility in Cattle—Is It a Cause for Concern? Animals, 12: 2654. https://doi.org/10.3390/ ani12192654.

Webb, L.E., Engel, B., Berends, H., van Reenen, C.G., Gerrits, W.J.J., de Boer, I.J.M. and Bokkers, E.A.M. 2014. What do calves choose to eat and how do preferences affect behaviour?. Applied Animal Behaviour Science. 161: 7-19. https://doi.org/10.1016/j.applanim.2014.09.016.

Zago, D, Canozzi, M.E.A. and Barcellos, J.O.J. 2020. Pregnant beef cow's nutrition and its effects on postnatal weight and carcass quality of their progeny. PLoS One. 27;15(8): e0237941. doi: 10.1371/journal.pone.0237941.

CHAPTER 5

Early-gestation maternal energy restriction in dairy heifers on gastrointestinal development, organ weight, behavior and digesta particle size distribution in post-weaned dairy calves

ABSTRACT

Dairy calves exposed to in utero maternal perturbations during pregnancy have shown to influence the development of their gastrointestinal tract later in life. Herein, this study tested the hypothesis that early gestation maternal energy restriction in dairy heifers will influence growth performance, feed intake, apparent total tract digestibility, and gastrointestinal organ morphology in their offspring at post-weaning. Briefly, Holstein Friesian heifers (n=28; BW±SD; 371.57±42.14, age±SD; 15.7±1.08 months) were divided into three groups based on body weight and age and, from 11 days before artificial insemination were individually fed as following: (i) 0.6 M of their maintenance energy requirements (M) until day 80 of gestation (nutrient restricted NR80, n = 11), (ii) 0.6 M until day 120 of gestation (NR120, n=11), and (iii) ad libitum (1.8 M) until day 120 of gestation (Control, n=6). Heifers were group fed once the individual feeding period ended, with ad libitum access to feed until calving. Twenty-two single female calves were retained after calving. All calves received the same management and diet until slaughter (BW±SD; 117±13). Four days until slaughter, calves were housed individually and received the same management and diet. Calves were euthanised at d 135±3 to obtain organs weight. Data was analysed in R with One-way ANOVA and mean contrast separated with Tukey post-hoc test. Body weight gain from weaning until slaughter remained similar among calves (P=0.959). Likewise, calves' stature evaluated was comparable among calves (P>0.1). Apparent total tract digestibility remained similar among all calves throughout the pre and post weaning phases. Although, while nutritional management during early gestation had no effect on calves ADG, slaughter weight, DMI, lower gut and foregut weights and total GIT weight, the weight of the duodenum in control calves tended to be heavier than their peers (P=0.07). This finding suggests that altering maternal energy in heifers during early gestation may impair duodenum development in their offspring. Nonetheless, these calves show the ability to catch up growth and maintain normal digestive physiology. Continued research is required to determine whether the lack of differences in most of the visceral organ weights or the tendency that duodenum development may be impaired will persist through production lifespan and generations.

Key words: Energy restriction, gastrointestinal tract, weaning, digesta, particle size distribution.

5.0. INTRODUCTION

Because the evidence is compelling that alterations in maternal nutrition during pregnancy may alter the long-term development and function of visceral organs in ruminants, a growing body of research in sheep (McGovern et al., 2015; Trotta and Swanson, 2021) and cattle (Duarte et al., 2013; Meyer and Caton, 2016) have focused on investigating maternal nutrition during different windows of pregnancy on visceral morphology, capacity, immunity, and functions in their progenies. In addition, other environmental stressors (e.g., heat stress during late gestation) have shown to alter the development of visceral organ in dairy calves (Reynolds et al., 2019; Dado-Senn et al., 2021; Wathes, 2022).

Programming of the gastrointestinal tract (GIT) is important because the GIT serves as the site for nutrient absorption and altering fetal GIT growth may permanently impair the capacity of an individual to efficiently utilize nutrient and thus, impair animal production efficiency (Wang et al., 2008). Furthermore, glycosidic linkages of saccharides that are digested by the enzyme's carbohydrase or glycohydrolase, are produced in the small intestine and pancreas (Trotta et al., 2020).

Importantly, at the commencement of enteral or postnatal feeding, the surface area and capacity of the GIT for digestion and absorption must be adequately developed (Trahair et al., 1997). This can be achieved if fetal organ growth is not compromised during fetal life as previous studies have shown that abnormal gastrointestinal tract (GIT) development is associated with compromised body growth *in utero* (Thornbury et al., 1993; Xu et al., 1994).

There is an unequal transfer of nutrients from the placenta to the embryonic organs (Zhang et al., 2021). Vital organs including the brain, heart, and liver receive priority than skeletal muscle and fat tissue throughout the first trimester (Long et al., 2009; Meyer et al., 2010).

However, long gestational mammals like cattle experience the most rapid phase of GIT development in the last trimester of gestation (Duarte et al., 2013; Modina et al., 2021). This perhaps supports historic assumptions that detailed dam's nutritional management is planned only in late gestation (McGovern et al., 2015). However, data from Duarte and colleagues (2013) have suggested that not all parts of the gastrointestinal tract is influenced by maternal nutrient restriction. What remains insufficiently understood is whether fetal GIT growth retardation from early gestation due to maternofoetal stress can predispose a long-term effect in adult life.

As previously stated, perturbations in maternal nutrition during gestation have been repeatedly used to model fetal programming in ruminants. Nonetheless, the bulk of studies on fetal programming from maternal nutritional stress on visceral organs have stopped at fetal life. Caton et al. (2009) reported greater fetal jejunum mass in overfed ewes than nutrient restricted ewes. In another study, crossbred cattle fed either 100% or 85% of their metabolic energy between 147 and 247 days after conception, showed no significant differences in the masses of placenta, heart, liver, kidney, or whole fetal body (Paradis et al., 2017). Fetal weight of total GIT, stomach complex, rumen, reticulum, and abomasum were not affected by either undernutrition or overnutrition regimens during mid pregnancy in beef cattle (Meyer et al., 2010). These studies to an extent explain the complexity in defining exact effects of maternal nutrition on visceral organ development.

A study on *in utero* heat stress in late gestation dairy heifers showed calves with impaired development of some key organs, however, all GIT organs evaluated at weaning were not different between calves from cooled mothers (Dado-Senn et al., 2021). The implication of this could support speculations that significant impact of maternal stress on GIT development may be missed at perinatal, especially when it has been reported that tissues with larger proportional mass is most evident at d 125 of pregnancy (Meyer et al., 2010) and that the GIT of a neonate has the capacity to grow in a catch-up manner (Trahair et al., 1997).

Despite encouraging results from fetal programming studies, *in utero* nutritional stress on organ morphology, growth, development, and function are still poorly understood. Growth factors and mediators of cell death control the development of organs and tissues, especially those engaged in physiological stress adaption (Penzo-Mendez and Stanger, 2015). It is believed that an increase in organ size will enable a larger calf to be supported, but it may also be linked to enhancements in organ growth, metabolic activity, and production (Kamiya et al., 2009; Geiger et al., 2016b).

Besides organ and phenotypic evidence of fetal programming, calves' behavior can also be reprogrammed. Feedlot calves from overfed cows during mid gestation had longer rumination time than their nutrient restricted counterparts, with similar management throughout postnatal life (Nascimento et al., 2022). On the other hand, recent evidence has suggested that calves preweaning feeding behavior particularly the number of rewarded or unrewarded visits to milk outlets and starter bunks is associated with long term performance of the calves (Swartz and Petersson-Wolfe, 2022). Thus, continued research in needed to associate developmental programming with behavioural epigenetics.

Knowledge on digesta particle size distribution is important as it is an important indicator of digestive physiology within the gastrointestinal tract in ruminants (Fritz et al., 2012; Clauss et al., 2016). For example, smaller fiber particles ferment more quickly than bigger fiber particles (Dufreneix et al., 2019).

Based on this, ruminants have finer digesta compared to other herbivores of similar body size (Clauss et al., 2015), and this finer digesta is achieved by the sorting mechanism, primarily, in the foregut (Lechner-Doll et al., 1991). Thus, finer digesta is expected in the lower gastrointestinal tract (Clauss et al., 2016). To achieve this, the sieve analysis is employed to identify variations in digesta particle distribution along the GIT, with a notable difference between the omasum and abomasum (because of their peculiarity) and minimal variations occurring in the lower tract of the GIT (Uden and Van Soest, 1982; Ahvenjärvi et al., 2001). To the best of our knowledge, there is no information available in literature on digesta particle size distribution in reprogrammed calves.

This chapter investigated the effects of maternal energy restriction in heifers during early gestation on the post weaning performance and organ weight and length of their offspring's gastrointestinal tracts, including the distribution of particle size digesta within each tract. The main hypothesis being tested was that exposure to *in utero* nutrient restriction in the first trimester of pregnancy will impair the development of the gastrointestinal tract in their offspring.

5.1. MATERIALS AND METHODS

Ethical procedures involving the use of animals have been reported in preceding Chapters.

5.1.1 Calves post-weaning management and fecal collection

Following the weaning phase, all calves were group housed in a single pen and fed at *ad libitum* levels until slaughter**.** Body weights and linear body measurements were monitored at three time points (d 103, 121 and 135 of age) before slaughter. Calves were provided with clean water and their bedding were cleaned regularly to prevent disease outbreaks.

Thirty days after weaning, feed and fecal samples were collected from the rectum of each calves into plastic containers at 6 time points (TP) for 3 consecutive days: TP1, d 1, 0800 h; TP2, d 1, 1600 h; TP3, d 2, 0900 h; TP4, d 2, 17:30 h; TP5, d 3, 1200 h; TP6, d 3, 1900 h, transported and stored at –20°C until analyzed. Fecal samples from TP1 to TP6 were mixed and a pooled sample were analyzed for DM, CP, NDF, starch and ADL estimates as previously described in Chapter 3

5.1.2 Behavioural observations

Sixteen calves were monitored during a 12-hour diurnal direct live observation. Two trained observers were positioned at least 15 m from the calves' boxes to ensure unobstructed views and avoid animal contact. Animals were observed at 3-min intervals, a duration which was shorter compared to the 10 mins interval duration adopted by Nascimento et al. (2022). Behavioural activities recorded and criteria used is similar to those reported in Chapter 3. A total of 11520 behavioural observations were recorded during the post-weaning period.

5.1.3 Slaughter protocol and organ collection

Four days until slaughter, calves were kept in single cages and fed a blended diet *ad libitum* (Table 5.1). Daily intake was recorded as feed offered minus orts. At ~ 24 h before calves were euthanized, body weight, thoracic circumference, hip height, back length and wither heights were recorded. All calves euthanized weighed (Mean±SD;117±13.1) and were 135±3 (Mean±SD) days old. Calves were transported to a local abattoir for evisceration. The calves were stunned with a high voltage first, then lacerated in the jugular artery to bleed them. The GIT, after collection, was freeze in a natural position to ensure that digesta does not freely move within organs. Total gastrointestinal tract was retrieved and immediately weighed. The rumen, omasum, abomasum, duodenum, ileum, jejunum, colon, caecum, rectum, Liver, spleen, kidneys, pancreas, and renal fats were dissected and weighed after euthanasia. The small and large intestines were transported back to the department of Agricultural Sciences, University of Sassari, Italy in iced portable fridge to measure organ length.

5.2 Calculations

Weight of organs was calculated by difference (total organ weight – empty organ weight). Dressing percentage was calculated as carcass weight divided by final live weight multiplied by 100. Dressing difference was calculated as live weight before slaughter minus carcass weight (Coyne et al., 2019). Organ length was measured with a tape meter as distance between one end (horn) and the other (bottom).

5.3 Particle size distribution of digesta

Procedure regarding particle size determination of digesta using wet sieving approach has been previously described (Silva, 2013; Clauss et al., 2016). The digesta samples in each component of the gastrointestinal tract from 8 calves was obtained regardless of maternal nutrition. The Endecotts wet sieving apparatus, which has seven sieves with pore sizes of 4.75, 3.35, 2.36, 1.18, 0.60, 0.30, and 0.15 mm, was used to determine the particle size distribution of the fed ration and gastrointestinal tract (reticulo-rumen, omasum, abomasum, duodenum, jejunum, colon, caecum, and rectum) contents samples. Contents in the Ileum were excluded as they did not meet the required quantity for sieving (Fujikura et al., 1989).

Particles retained on sieves smaller than 1.18 mm were categorized as small (PP), while those retained on sieves bigger than 1.18 mm were categorized as large (PG) (Seo et al., 2009). About 25g of the fed ration sample and 200g of digesta were sieved for 10 minutes at the recommended water flow rate of 1.5 liters/minute for the device. Before sieving the dry samples (feed and orts), they were immersed in 330 mL of water for 20 minutes to break the surface tension of the water particles.

After sieving, the material retained on each sieve was collected on aluminium plates of known weight, taken to a 105°C oven for 24 hours and, later weighed to determine the distribution of dry matter on the sieves. The results were expressed as a proportion of the particle size retained on each sieve and expressed as a percentage dry matter of fresh matter. Mean particle size (MPS) was expressed as discrete mean (dMEAN) according to the formula reported by Clauss et al. (2016):

$$dMEAN = \sum_{i=1}^{n} p(i) * \frac{s(i+1) + s(i)}{2}$$

Where p(i) is the proportion particle, s(i) mesh size number and n are the maximum particle size measured manually. Detailed illustrious calculation of dMEAN has been previously reported by Fritz et al. (2012).

By dividing the offered and consumed amounts of small and large particles, the selectivity index (SI) was determined. SI values above 1 indicate that the animal chose a certain portion of the diet, whereas SI values below 1 indicate that the animal declined that portion of the diet. When SI was 1, there was no selection or rejection; instead, the animals consumed everything that was presented to them (Jamieson and Hodgson, 1979).

5.4 Data analysis

All dependent variables were screened for normality using the Shapiro wilk test in R (R Core Team, 2020). Once the normality test was passed, the data was analysed as a linear model with One-way ANOVA. The Tukey's test for honestly significant differences was used to separate the least squares means when the predictor effects were deemed significant. Significance was claimed for $P<.05$ and a tendency was declared when $P>0.05<0.1$.

5.5. RESULTS

5.5.1 Growth performance and Intake

Figures 5.1A-F shows body weight gain and other body linear measurements of calves during the post-weaning phase. Despite differences in birth weight of calves, no difference in both weaning and post weaning weights was observed ($P>0.1$). Furthermore, no interaction effect was detected between experimental groups and age post weaning ($P>0.1$). While age effect was found to be significant for all body measurements ($P<0.05$), treatment and interaction effects remained similar ($P>0.1$) for back length, hip height, heart girth and average daily gain. Treatment effect tended to differ ($P=0.105$) for wither height with control calves showing tendency to be taller than their peers (Figure 5.1C).

Despite of the absence of gross difference in average daily gains among calves, all calves experienced a marked decline ($P<0.0001$) in gains between weaning age (d 74) and post weaning age (d 121) where calves gained ~ 500g/d (Figure 5.1F).

Dry matter intake prior to slaughter (Figure 5.2) was similar among calves ($P = 0.754$). Calves consumed more than 3000g of blended feed during this phase.

5.5.2 Behavioural Observation

Behavioural patterns of weaned calves are presented in Table 5.2. Daughter calves from NR80 fed heifers spent the most time eating compared to calves from control and NR120 fed heifers, respectively ($p<0.0001$). However, time spent ruminating was similar for calves from Control and NR80 fed heifers and higher than NR120 fed heifers ($P=0.006$). Since NR120 calves spent the least time ruminating and feeding, they spent the most time lying down ($P<0.0001$), while time spent carrying out other activities remained similar among all calves ($P=0.338$). As expected, hourly behavioural activities differed significantly ($P<0.0001$) during the 12 h period (Appendix 5.1A-H).

5.2.3 Organ weights and lengths

The weights of several organs and carcass performance are displayed in Table 5.3. The low birth weight of NR80 calves was not associated to their slaughter weight ($r=0.24$, $P=0.597$), nor was there a relationship between heavier calves at birth and their slaughter weight ($r=0.49$, $P=0.395$). Furthermore, there was no observed difference in weights of carcass, spleen, pancreas, and total GIT ($P>0.1$). However, there tended to differ in renal fat weight ($P=0.14$). Although there was an association between carcass weight and dressing difference ($r=0.77$, $P=0.0001$), a moderate linear relationship was observed between these two variables (Figure 5.3).

Organs expressed as grams per kg of slaughter live weight remained similar among all calves, most likely because live slaughter was not significantly different ($P>0.1$).

Fore gut digestive organs were similar ($P>0.1$) among all calves (Table 5.4). Even when expressed based on grams per kg liveweight at slaughter, this effect was not eliminated ($P>0.1$).

Weight of duodenum (P=0.078), ileum (P=0.15), and rectum (P=0.13) tended to differ in daughter calves (Table 5.5). Calves from control fed heifers tended to have higher duodenum and rectum weight than calves from nutrient restricted mothers. For ileum weight, NR80 calves showed higher numeric weights compared to calves from Control and NR120 fed heifers. Also, when these organ weights were expressed based on grams per kg slaughter live weight, the higher numeric difference persisted.

Despite the tendency for duodenum and ileum weights to differ across treatments, no association was found between duodenum and slaughter weight (r=0.02, P=0.918) or between Ileum and slaughter weight (r=0.18, P=0.424) in calves. Furthermore, no association was found between low birth weight of NR80 calves and duodenum weight (r=-0.07, P=0.868). Equally, no association was found between birth weight in heavier calves and duodenal weight (r=-0.37, P=0.536).

The length of the small and large intestine is presented in Table 5.6. No organ length difference was observed in the small intestine across treatments (P>0.1). However, there tended to differ in caecum length (P=0.17) as calves from control fed heifers showed a tendency of shorter length compared to their peers. Both caecum and rectum lengths, expressed on percentage basis of slaughter live weight, tended to be shorter in control calves than their peers (P<0.08).

5.5.4 Apparent total tract digestibility

No difference (P>0.1) in dry matter, crude protein, starch and NDF apparent total tract digestibility was observed (Table 5.7). However, when apparent digestibility was evaluated between weaning and post weaning, DM, NDF and starch digestibility increased after weaning in all calves irrespective of their maternal nutrition during pregnancy (P<0.0001).

5.5.5 Digesta particle size distribution

In Figure 5.4, the frequency interpretation of sieve analysis of digesta particle size distribution along the gastrointestinal tract is presented. In the foregut, rumen digesta had higher particle sizes evidenced on large sieve mesh. On 0.15mm sieve size, the abomasum had higher mean particle size expressed on dry matter basis. Lower particle sizes were observed in the small intestine on large sieves; however, the highest proportion of particles was found on the small sieve mesh with digesta particles in the duodenum higher than those in the jejunum. Interestingly, particle proportions in the jejunum were higher in bigger sieves. Similarly, higher mean particles sizes were present in the smaller sieves (0.6-0.15mm) than the larger ones for digesta found in the large intestine. As expected, on smaller sieves, higher particle proportions were found in the rectum compared to those in the caecum or colon.

Dry matter (DM) distribution, expressed as percentage of fresh weight (Figure 5.5A), along the gastrointestinal tract varied significantly (P<0.0001). Rumen digesta had higher dry matter content, with DM contents in the abomasum slightly higher than those in the omasum. Lower DM concentration was detected in the Jejunum while elevated DM content was observed in the caecum and colon, while DM in the rectum appeared to be lower.

Because dMEAN have been proposed as the standard for the description of mean particle size value obtained from sieve analysis, the overall average MPS obtained from wet sieving is presented in Figure 5.5B. Larger dMEAN was observed in the rumen and abomasum while the lower tract recorded smaller dMEAN (Figure 5.5.B).

Based on the selectivity index estimation, calves selected smaller feed particles than larger ones (Figure 5.6). However, except for the rumen, comparing ingested particles with digesta particle distribution along the GIT, showed a marked difference with higher digesta particle proportions retained on smaller sieves (Figure 5.7). Ingested particle proportions were higher predominantly in the 4.75mm large sieve. This ascendancy disappeared between sieve size 1.18 and 3.35mm.

5.6. DISCUSSION

Changes in maternal nutrition during pregnancy have been at the center of a lot of recent studies due to the ongoing interest in understanding the concept of fetal or developmental programming using animal models. Dairy cattle that are subjected to in utero environmental stress *in utero,* show altered growth, immunity, and productivity, which raises animal welfare concerns and is detrimental to farm economy (Tao et al., 2019; Ahmed et al., 2021). For instance, postnatal immunological functioning and physiology are altered in calves with late gestation *in utero* heat stress (Dahl et al., 2016). The impact of the prenatal dietary environment on the postnatal growth, development, and function of fetal organs as well as the distribution of digesta particle size along the gastrointestinal tract in dairy calves is less well known. Herein, this study investigated how a calf's organ and body growth could be impacted by nutritional changes that take place during pregnancy, perhaps resulting in early developmental issues. The results of the present study and those reported in Chapter 4 confirm and demonstrate that *in utero* nutritionally stressed heifers are smaller in weight, stature, and other body size measurements at birth while the weights of the small intestinal organs (duodenum and ileum) tended to be underdeveloped compared to their counterparts when euthanized at d 135 postnatal. Additionally, calves showed digesta particle sizes that varied along the gastrointestinal tract, regardless of the maternal nutrition during early gestation, indicating normal digestive physiology of the digestive system.

In the current study, there is evidence of impairment of the small intestine in calves born to nutrient restricted mothers. Young animals including calves, are susceptible to high rates of mortality and morbidity in the neonatal period and this problem is further aggravated by preterm birth (Flemming and Nielsen, 2001). Organ immaturity and dysfunction particularly of the GIT (Sangild et al., 2000) and the immune system (Barrington and Parish, 2001) can cause early life losses. Neonatal calves are therefore highly vulnerable to insults that could decrease the transfer of colostral immunoglobulins because passive uptake of immunoglobulins is the only immunoprotective mechanism in the bovine (Barrington and Parish, 2001). Comparative data have shown that the small intestine responds to maternal nutrient restriction by expanding its surface area (Duarte et al., 2013). However, these authors further reported that not all of the fetal immunological and digestive organs are altered throughout pregnancy (Duarte et al., 2013), which aggress with data from the current study.

Gastrointestinal tissues grow significantly during the perinatal period, despite the fact that organ and fetal growth accelerates in the late stages of pregnancy (Lyford, 1988). This indicates that a significant percentage of the extremely rapid late gestational growth in visceral organ size, particularly in the organs with bigger proportional mass at d 125 of gestation (Meyer et al., 2010), may have been missed since in the current study, organs were collected d 135 of the calves' post-natal life. Furthermore, the vast majority of research examining the visceral GIT and immunological organs of *in utero* perturbed cattle have assessed these organs at birth or at weaning. We could speculate that severe impact of IUGR of important visceral organs can be immediately seen at birth or between birth and weaning.

In addition, some visceral organs like the rumen continues to rapidly develop from the juvenile stage to complete establishment, aided by increased solid or dry feed during the preweaning management (Khan et al., 2011; Diao et al., 2019), which lends further credence to the aforementioned claim. Also, the maternal gastrointestinal system reacts to both nutritional condition and pregnancy status throughout gestation (Reed et al., 2007). This information suggests that the dam might compensate for nutritional losses, preserving her offspring and any maldevelopment of most visceral tissues may not be visible after birth.

Feeding and behavioural patterns in calves continued to differ between pre and post weaning phases in the current study. Although control calves showed equal rumination time compared with calves from nutrient restricted mothers, results obtained from this study was not consistent with those reported by Nascimento et al. (2022) that reported at d 100 of life, progenies in the control group (10% CP supplementation in late gestation) spent 52% more time per day eating supplements than their peers, and at d 210, they spent 17% less time being inactive. In the feedlot period, the males from the control group also ruminated for 15 minutes longer each day than their counterparts (Nascimento et al., 2022). However, it is important to note that the breed, period, and type of maternal nutritional intervention as well as the offspring's sex and management system reported in the study by Nascimento, and colleagues (2022) contributes to the differential results obtained in the current study.

The fact that in the current study, the duodenum tended to be underdeveloped in the nutrient restricted calves could suggest some sort of reprogramming epigenetics, and this has been previously reported in fetal bovine (Khanal et al., 2020).

The importance of the duodenum cannot be overemphasised as it is the first part of the small intestines where absorption of nutrients begins due to its ability to receive partially degraded food from the stomach (Akonyani et al., 2021). The duodenum, which represents the small intestine's largest diameter, densest villi, and deepest portion, is where degradation occurs closest to the point of consumption. Further, the pancreas and liver create bile, which the duodenum absorbs. This helps the intestines break down fat, protein, and carbohydrates (DeGregorio et al., 1982; Meyer and Caton, 2016). In addition, the absorbed secretions make it possible for the small intestine to neutralize the low pH digesta coming from the rumen, thus creating an enabling environment for intestinal enzymes to thrive. Negatively, assuming the propensity for the low duodenal weight in the nutrient restricted calves persists throughout life, this is a potential for poor digestion and absorption of nutrients and consequently reduces the performance efficiency of the animals in the herd.

According to Dado-Senn et al. (2020a), due to poor intestinal growth and a reduction in the surface area for absorption, impaired passive transfer in prenatal heat exposed calves may be caused by decreased postnatal absorption in the small intestine. In the current study, calves conceived by nulliparous heifers and subjected to *in utero* nutrient restriction may have impaired small intestine development and function more so than in any other growth parameter evaluated.

In contrast, the length of the caecum, expressed on % slaughter live weight, tended to be impaired in control calves than their peers. While it has been previously reported that intestinal length, rather than mass is a better predictor of IUGR (Trahair et al., 1997), it is not completely surprising that control calves showed tendency for impaired organ. Comparative studies have shown that maternal perturbations during pregnancy on visceral organ development is inconsistent. However, the drawback of using this metric (intestinal length) is that it requires a complete outlook on the geometry and diameter of the intestinal mucosa as well as changes in both villi and microvilli (Trahair et al., 1997). However, this was not evaluated in the present study.

Calves' digestive physiology evaluated through digesta physical structure and particle size distribution along the GIT, showed no dissimilarities compared to what is obtainable in a typical ruminant as finer particle sizes were present in the lower gut compared to the upper gut, and higher dry matter content observed in the foregut than in the small intestinal and thereafter, elevated DM content in large intestine particularly between the colon and rectum. This has been previously reported in grass or browse goats (Clauss et al., 2016). However, digesta buoyancy and or density that indicate a low concentration of digesta particles in the small intestine and occasionally longer particle size in the same organ may be caused by the foregut's sorting system, notably the reticulum (Lauper et al., 2013). Because the morphophysiological system of a ruminant can be thought of as a pool from which some qualities for breeding programs in domestic ruminants can be selected, the information on digesta particle distribution in the current study can be an important proxy to normal digestive physiology (Han et al., 2021) even though there is indication of impaired duodenum development in calves born to nutrient restricted mothers during early gestation.

Because this study was carried out on a commercial farm, there is a lack of total control over dam early-life (periconception) and calf postweaning events. According to earlier research (McGovern et al., 2015), late gestational *in utero* nutritional stress has an immediate impact on organ development from birth to weaning. As a result, potential differences in the dams' early life (prior to breeding) or during the postweaning life of the calves could have affected the offspring organ outcomes in the present study. In addition, despite efforts to provide a postnatal homogenous, thermoneutral environment and nutritional management, it is still possible that exposure to additional stressors (beyond the purview of this study) occurred during pregnancy and after birth and may have affected the calf physiology and feeding behaviours recently documented by Dado-Senn et al. (2020c) in an in-utero heat stress study.

5.7. CONCLUSION

In light of the current findings, data showed that calves develop the ability to undergo catch up growth, which is demonstrated by comparable body weight, stature, dry matter intake, and foregut organ weight among calves between weaning and slaughter. However, in partial agreement with the hypothesis of the current study, the tendency for the duodenum to weigh less in calves from early gestation nutrient restricted mothers suggests that this organ development may be impaired with a potential setback for the secretion of enzymes necessary to break down glycosidic linkages of saccharides to enhance nutrient digestion and absorption in the lower gut. Positively, calves demonstrated normal and typical digestive physiology in the gastrointestinal system regardless of their maternal nutrition during early gestation, where high concentration of smaller particles in the lower gut and a noticeable variation in particles between the omasum and abomasum were detected.

Table 5.1. Chemical composition of experimental diet (DM basis)

Item	Composition (%)
DM	92.4
CP	12.4
NDF	50.3
ADF	39
ADL	6
EE	1
Ash	8.7
ADIP	1.3
Sugars	11.4
SOLP	5.6
NDIP	3.6
NDF24	46.2
Starch	8.3
[1]NFC	27.6
[2]ME (Mcal/kg)	2.12

[1]Calculated as NFC = 100 − CP − ash − NDF − ether extract.

[2] Calculated using NRC, 2001 guidelines.

NDIP= neutral detergent insoluble protein, ADIP= acid detergent insoluble protein, SOLP= soluble protein. Blended feed composed of hay, soya and minerals (Ca = 0.5%, P = 0.3%, Mg = 0.2%, K = 2.2%)

Table 5.2. Behavioural patterns of post weaned calves during a 12 h diurnal period

Item (mins/12h)	Treatment			SEM	p-value
	Control (n=4)	NR120 (n=6)	NR80 (n=6)		
Feeding	^{b}168	^{c}137	^{a}195	0.007	<0.0001
Rumination	^{a}240	^{b}220	^{a}241	0.007	0.006
Resting	^{b}184	^{a}224	^{c}152	0.007	<0.0001
Others	129	139	132	0.006	0.338

a–c Means within a column with different superscripts differ (P ≤ 0.05), Others = combination of activities including drinking, vocalization, walking and standing without eating. Calves belonged to mothers fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

Table 5.3. Performance and organ weights of post weaned dairy calves born to nutrient restricted or overfed mothers during early gestation (Mean±SE)

Item	Treatment			r	p-value
	Control (n = 5)	NR120 (n = 9)	NR80 (n = 7)		
Slaughter live weight (kg)	118±3.4	119.4±3.3	117.8±6.4		0.951
BCS	2.8±0.04	2.9±0.04	2.9±0.04		0.233
Carcass weight (kg)	52.6±2.6	53±2.1	52.9±3.3	0.95	0.998
Dressing (%)	44.5±1.1	44.3±0.8	45±1.1	0.45	0.842
Total GIT (kg)	35.7±1.2	35.1±0.8	35±1.0	0.56	0.938
Total GIT (%)	30.3±1.1	29.6±0.9	30.7±1.3	-0.73	0.756
GIT g/kg BW	302.8±10.7	295.8±9.8	304.7±14.7	-0.73	0.846
Spleen (g)	265.2±14.3	243.7±13.3	251.6±21.2	0.81	0.693
Spleen (%)	0.2±0.009	0.2±0.008	0.2±0.008	0.27	0.259
Spleen (g/kg BW)	2244.2±91.2	2032.7±80	2125.1±90	0.27	0.283
Pancreas (g)	132.2±12.4	129.4±9.7	142.9±13.7	0.61	0.692
Pancreas (%)	0.1±0.009	0.1±0.006	0.1±0.008	0.12	0.540
Pancreas (g/kg BW)	1118.5±89.3	957.7±131.5	1217.8±90.7	0.11	0.269

Table 5.3 Continued

Item	Treatment			r	p-value
	Control (n = 5)	NR120 (n = 9)	NR80 (n = 7)		
Tongue (%)	0.4±0.01	0.4±0.01	0.4±0.01	-0.59	0.989
Liver/Kidney/Esophagus (g)	3947.1±848.7	5650.5±1909	3308±366.5	-0.59	0.454
Liver/Kidney/Esophagus (%)	3.4±0.7	4.6±1.5	3.1±0.5	-0.05	0.562
Renal fat (g)	1567.3±888	554.1±78	601.2±86.9	0.01	0.141
Renal fat (%)	1.3±0.8	0.5±0.03	0.5±0.1	-0.03	0.186

g/kg BW = calculated based on slaughter live weight. Calves belonged to mothers fed; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation. NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. r = Association between organs and slaughter live weight. % Organ weight was calculated based on slaughter live weight.

Table 5.4. Weight of foregut organs of post weaned dairy calves born to nutrient restricted or overfed mothers during early gestation (Mean±SE)

Item	Treatment			r	p-value
	Control (n = 5)	NR120 (n = 9)	NR80 (n = 7)		
Rumen (kg)	15.8±0.9	16.9±0.6	17.6±0.7	0.14	0.299
Rumen (g/kg BW)	134.6±8.3	143±7.1	152.9±11.5	-0.62	0.444
Rumen (%)	13.5±0.8	14.3±0.7	15.2±1	-0.62	0.451
Omasum (g)	991.7±118.8	975±123.7	1140.7±144.3	0.61	0.627
Omasum (g/kg BW)	8323.6±797.7	8070.1±926.6	9710.3±1037.1	0.37	0.441
Omasum (%)	0.8±0.08	0.8±0.09	0.9±0.1	0.37	0.796
Abomasum (g)	976.1±182.3	974.2±151.7	1046.1±283.4	0.35	0.963
Abomasum (g/kg BW)	8170.7±1342	8325±1377.5	8595.2±2102	0.12	0.986
Abomasum (%)	0.8±0.1	0.8±0.1	0.9±0.2	0.13	0.990

g/kg BW = calculated based on slaughter live weight. Calves belonged to mothers fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. r = Association between organs and slaughter live weight. % Organ weight was calculated based on slaughter live weight

Table 5.5. Organ weight of small and large intestines of post weaned dairy calves born to nutrient restricted or overfed mothers during early gestation (Mean±SE)

Item	Treatment			r	p-value
	Control (n = 5)	NR120 (n = 9)	NR80 (n = 7)		
Duodenum (g)	87.9±23.8	47.3±9.4	43±9.3	0.02	0.078
Duodenum (%)	0.1±0.02	0.01±0.008	0.01±0.01	-0.12	0.09
Duodenum (g/kg BW)	748.9±200.9	394.7±79	374.5±96	-0.11	0.090
Ileum (g)	38.7±10.5	31.3±7.6	59.7±13.6	0.23	0.153
Ileum (%)	33.8±10.2	29.6±6.6	49.2±8.3	0.02	0.198
Ileum (g/kg BW)	338±101.7	266.8±65.2	492.5±96.2	0.02	0.16
Jejunum (g)	2762.4±376.9	2864.7±158.5	2667.4±272	0.33	0.838
Jejunum (%)	2.3±0.3	2.4±0.1	2.4±0.2	-0.24	0.969
Jejunum (g/kg BW)	23344.3±2971	24080±1354.5	22800.3±1982.4	-0.24	0.884
Total small intestine (g)	2888.9±357	2943.3±162.1	2770.1±280	0.35	0.871
Total small intestine (%)	2.4±0.3	2.5±0.1	2.5±0.2	-0.24	0.994
Colon (g)	1324±200.8	1469.7±148.4	1584.2±254.9	0.14	0.711
Colon (%)	1.1±0.2	1.2±0.1	1.4±02	-0.21	0.434
Colon (g/kg BW)	11160.5±1532.4	12318.1±1135.3	13556.4±2224.6	-0.21	0.650

Table 5.5. Continued

Item	Treatment			r	p-value
	Control (n = 5)	NR120 (n = 9)	NR80 (n = 7)		
Caecum (g)	740.4±142.7	739.8±65.9	813.4±85.5	-0.09	0.804
Caecum (%)	0.6±0.1	0.6±0.06	0.7±0.08	-0.46	0.489
Caecum (g/kg BW)	6351.7±1317.6	6219.7±556	7025.6±787	-0.46	0.746
Rectum (g)	264.4±37.3	112.2±28.2	165.9±71.7	-0.12	0.137
Rectum (g/kg BW)	2227.9±292	944.9±239.7	1517.8±680	-0.22	0.177
Rectum (%)	0.2±0.03	0.1±0.02	0.1±0.06	-0.22	0.169
Total large intestine (g)	2328.8±276.7	2321.7±195.6	2563.6±364	0.05	0.786
Total large intestine (%)	2±0.2	1.9±0.2	2.3±0.3	-0.34	0.451

g/kg BW = calculated based on slaughter live weight. Calves belonged to mothers fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. % Organ weight was calculated based on slaughter live weight. r = Association between organs and slaughter live weight

Table 5.6. Length of small and large intestines of post weaned dairy calves born to early gestation nutrient restricted or overfed mothers (Mean±SE)

Item (cm)	Treatment			r	p-value
	Control (n = 5)	NR120 (n = 9)	NR80 (n = 7)		
Duodenum	51±9.5	55±10.1	57.9±10.8	-0.33	0.915
Duodenum (%)	0.4±0.009	0.5±0.08	0.6±0.11	-0.59	0.588
Ileum	32.1±5.4	35.3±6.3	29.1±2.7	-0.28	0.719
Ileum (%)	0.3±0.06	0.3±0.06	0.3±0.05	-0.56	0.939
Jejunum	2700.8±190.8	2739.1±151.5	2671.9±100.1	0.31	0.939
Jejunum (%)	22.9±1.9	23.3±1.3	23.8±1.1	-0.59	0.920
Caecum	32.4±1.2	40.5±2.2	42.3±4.3	0.25	0.169
Caecum (%)	0.3±0.01	0.3±0.02	0.3±0.02	-0.23	0.101
Colon	442.5±28.2	459±22.5	430.4±28.6	0.21	0.724
Colon (%)	3.7±0.18	3.9±0.16	3.9±0.3	-0.58	0.939
Rectum	44.5±1.4	47.1±4.4	53.1±5.1	-0.21	0.345
Rectum (%)	0.4±0.02	0.4±0.04	0.5±0.07	-0.59	0.188

Calves belonged to mothers fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation. % Organ weight was calculated based on slaughter live weight. r = Association between organs and slaughter live weight

Table 5.7. Apparent total tract digestibility in post-weaned female dairy calves born to early gestation nutrient restriction or overfed mothers (Mean±SE)

Item (%)	Treatment Control (n=5)	 NR120 (n=9)	 NR80 (n=8)	p-value
DM	79.7±0.2	80.2±0.5	79.8±0.5	0.729
CP	77.9±3.2	80.2±2.2	78.9±2.6	0.810
NDF	70.8±1.5	70.5±0.8	70.8±0.8	0.970
Starch	98±0.2	98.2±0.2	98.3±0.3	0.579

Calves belonged to mothers fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

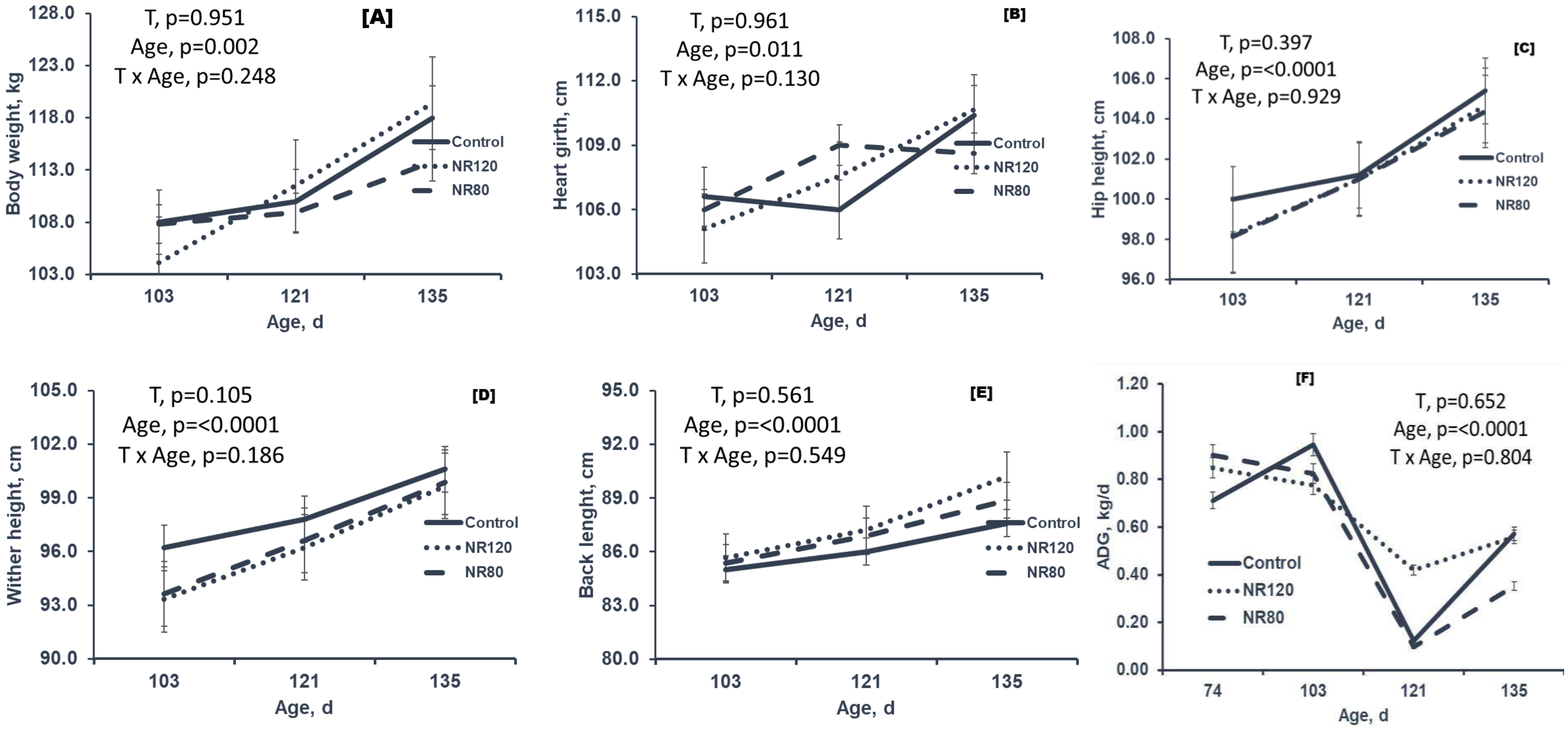


Figure 5.1. Body weight and linear measurements of post weaned calves (n=22). Calves were born to NR80=0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120=0.6 M until day 120 of gestation and Control=ad libitum (1.8 M) until day 120 of gestation.

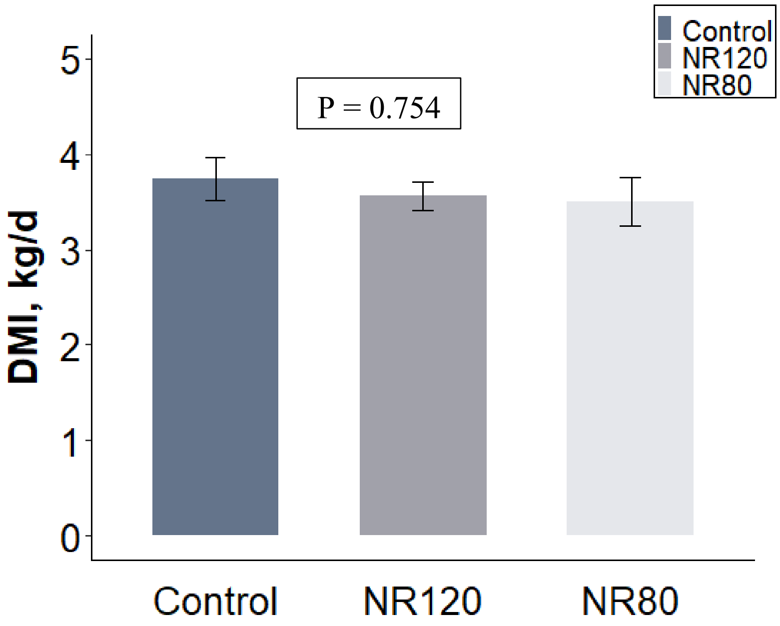


Figure 5.2. DMI of post weaned calves (n=22) during the last 4 days to slaughter. Error bars represent standard error of mean. Calves belonged to mothers fed either; NR80 = 0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120 = 0.6 M until day 120 of gestation and Control = ad libitum (1.8 M) until day 120 of gestation.

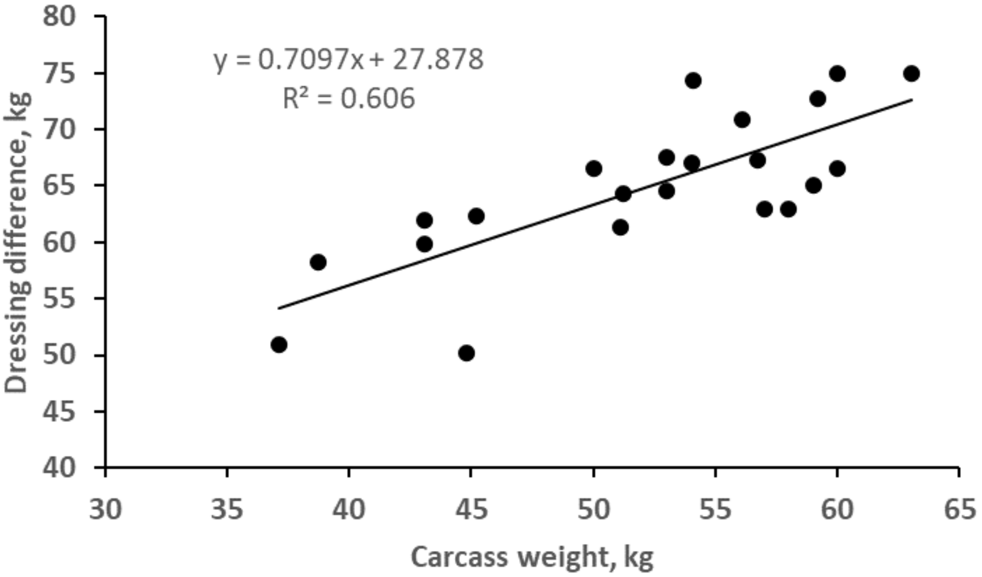


Figure 5.3. Linear relationship between carcass weight and dressing difference of calves (n=22). Linear association was calculated independent of calves' maternal nutrition during pregnancy. Dressing difference was calculated as live weight before slaughter minus carcass weight.

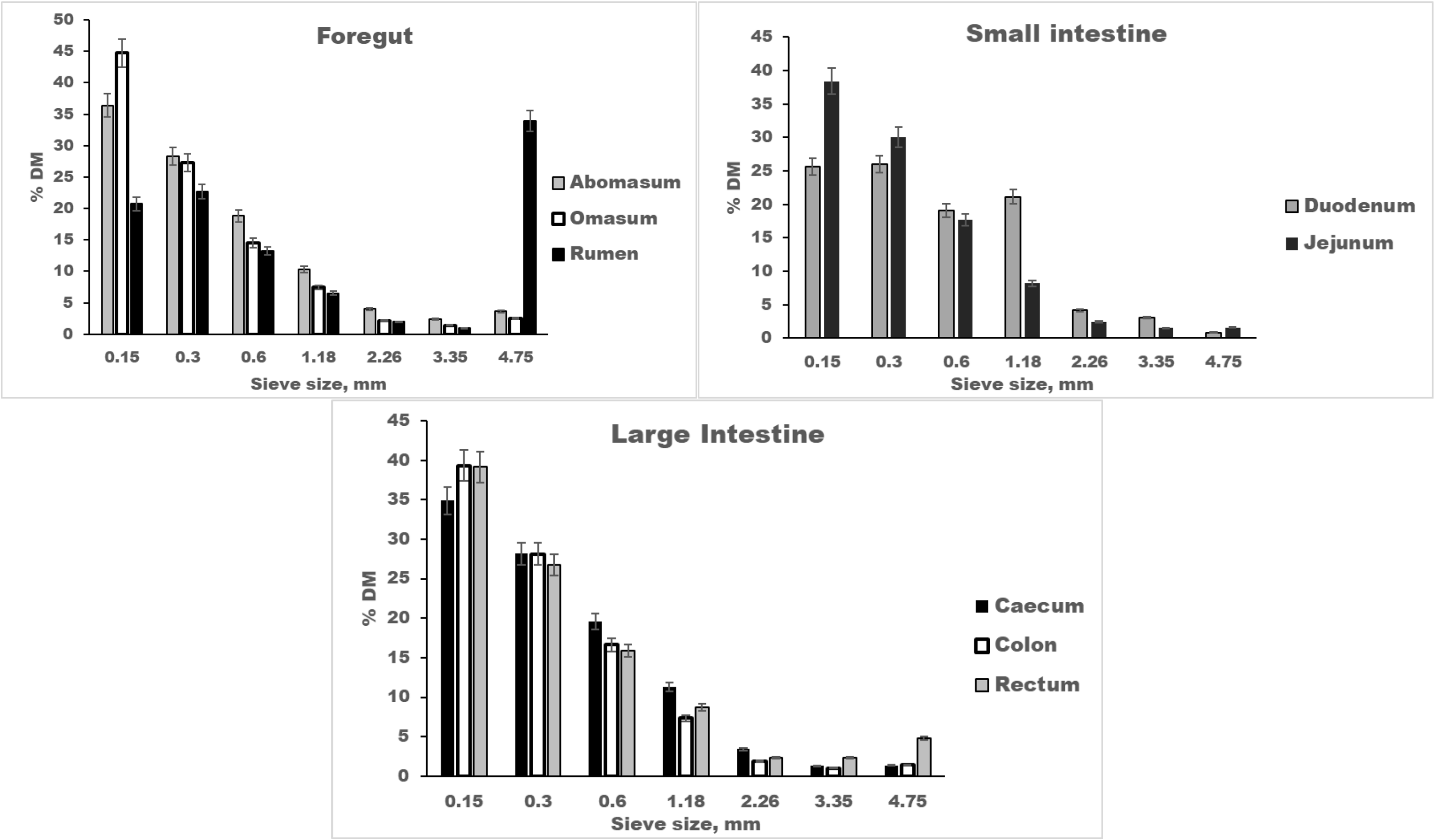


Figure 5.4 Sieve analysis of dry matter digesta particle distribution along the gastrointestinal tract in calves (n=8). Digesta were sieved on sieve mesh with sizes ranging from small (0.15mm) to big (4.75mm) sieve sizes. Higher percent of finer digesta particles were retained in small sieve sizes particularly in the lower gut. Larger digesta particle in the rumen were retained on bigger sieve sizes.

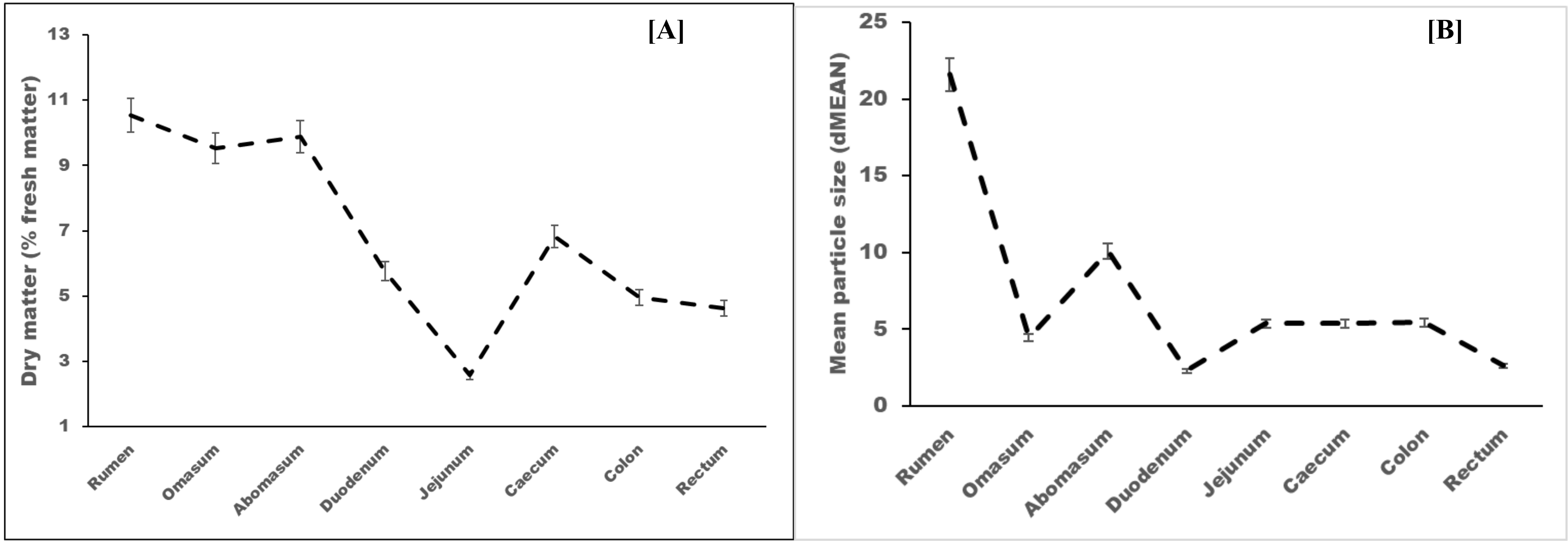


Figure 5.5.a-b Dry matter distribution (expressed as a percentage of fresh weight) and mean particle size (dMEAN) of digesta along the gastrointestinal tract in calves (n=8). Calves sampled in this study were randomly selected irrespective of their maternal nutrition during pregnancy.

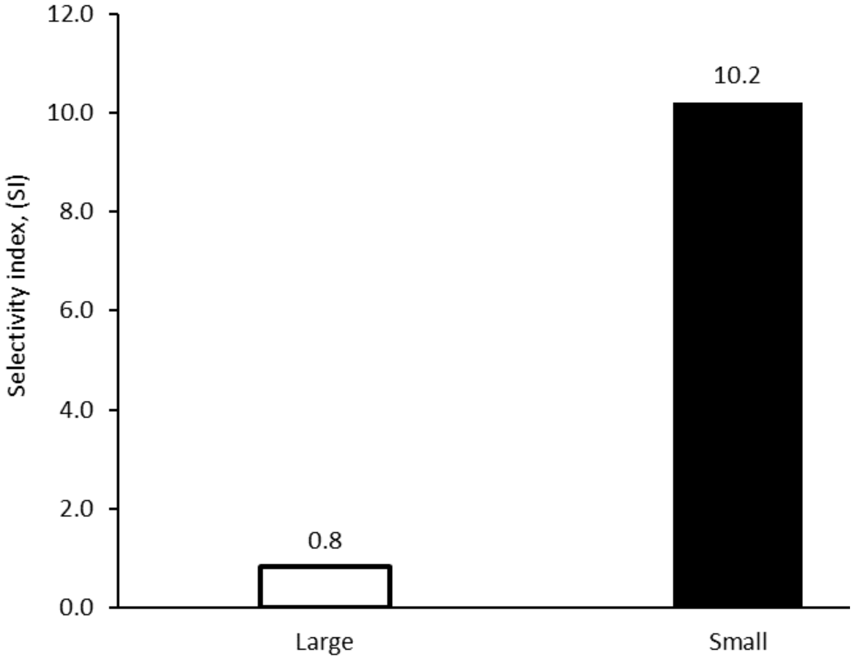
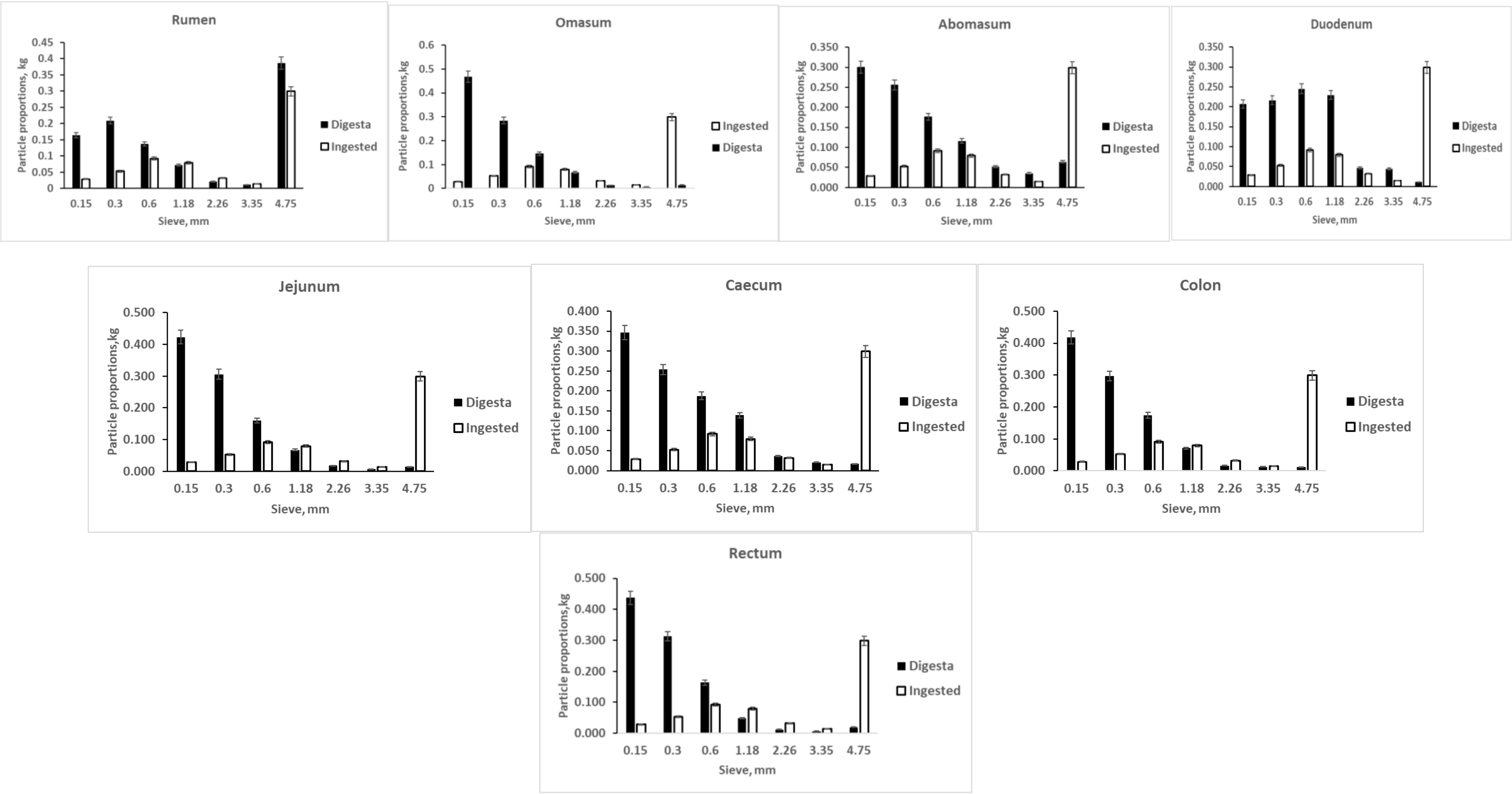


Figure 5.6. Selectivity index of ingested feed calculated by dividing the ingested and offered, for small and large particles. SI above 1 means that the animal selected a certain fraction of the diet, below 1 represents that the animal did not and there was refusal by that particle size. For SI equal to 1, there was no selection or refusal, i.e., everything that was offered was consumed by the consumed by the animals.



Rumen

Omasum

Abomasum

Duodenum

Jejunum

Caecum

Colon

Rectum

Figure. 5.7. Sieve analysis to compare the particle proportions (%) between ingested feed and digesta obtained from the different gastrointestinal tract in calves (n=8).

REFERENCES

Ahmed, B.M.S., Younas, U., Asar, T.O., Monteiro, A.P.A., Hayen, J., Tao, S. and Dahl G.E. 2016. Maternal heat stress reduces body and organ growth in calves: Relationship to immune tissue development. J. Anim. Sci. 94(Suppl. 5):617. doi: 10.2527/jam2016-1279.

Ahvenjärvi, S., Skiba, B. and Huhtanen, P. 2001. Effect of heterogeneous digesta chemical composition on the accuracy of measurements of fiber flow in dairy cows. Journal of Animal Science. 79: 1611-1620.

Akonyani, Z.P., Song, F., Li, Y., Qiqige, S. and Wu J. 2021. Comparative Analysis of the Microbiota Between Rumen and Duodenum of Twin Lambs Based on Diets of Ceratoides or Alfalfa. Pol. J. Microbiol. 70(2):175-187. doi: 10.33073/pjm-2021-015.

Barrington, G. M., and S. M. Parish. 2001. Bovine neonatal immunology. Vet. Clin. North Am. Food Anim. Pract. 17:463–476. https://doi.org/10.1016/S0749-0720(15)30001-3.

Caton, J.S., Reed, J.J., Aitken, R.P, Milne, J.S., Borowicz, P.P., Reynolds, L.P., Redmer, D.A. and Wallace, J.M. 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. Journal of Animal Science.87(1):222-35. doi: 10.2527/jas.2008-1043.

Clauss, M., Fritz, J., Tschuor, A., Braun, U., Hummel, J. and Codron, D. 2017. Dry matter and digesta particle size gradients along the goat digestive tract on grass and browse diets. Journal of Animal Physiology and Animal Nutrition 101 (1): 61. https://doi.org/10.1111/jpn.12505.

Coyne, J.M., Evans, R.D. and Berry, D.P. 2019. Dressing percentage and the differential between live weight and carcass weight in cattle are influenced by both genetic and non-genetic factors1. Journal of Animal Science. 97(4):1501-1512. doi: 10.1093/jas/skz056.

Dado-Senn, B., Field, S.L., Davidson, B.D., Casarotto, L.T., Marrero, M.G., Ouellet, V., Cunha, F., Sacher, M.A., Rice, C.L., Maunsell, F.P., Dahl, G.E. and Laporta, J. 2021. Late-Gestation in utero Heat Stress Limits Dairy Heifer Early-Life Growth and Organ Development. Frontiers in Animal Science. 2:750390. doi: 10.3389/fanim.2021.750390.

Dado-Senn, B., Laporta, J. and Dahl, G.E. (2020a). Carry over effects of late-gestational heat stress on dairy cattle progeny. Theriogenology 154:17-23. doi: 10.1016/j.theriogenology.2020.05.012.

Dado-Senn, B., Vega Acosta, L., Torres R.M., Field, S.L., Marrero, M.G., Davidson, B.D., Tao, S., Fabris, T.F., Ortiz-Colon, G., Dahl, G.E. and Laporta, J. (2020c). Pre- and postnatal heat stress abatement affects dairy calf thermoregulation and performance. J. Dairy Sci. 103: 4822-4837. doi: 10.3168/jds.2019-17926.

Dahl, G. E., Tao, S. and Monteiro, A.P.A. 2016. Effects of late-gestation heat stress on immunity and performance of calves. Journal of Dairy Science. 99:3193-3198. https://doi.org/10.3168/jds.2015-9990.

DeGregorio R.M, Tucker, R.E., Mitchell, G.E. Jr, and Gill, W.W. 1982. Carbohydrate fermentation in the large intestine of lambs. Journal of Animal Science. 54(4):855–862.10.2527/jas1982.544855x.

Diao, Q., Zhang, R. and Fu, T. 2019. Review of Strategies to Promote Rumen Development in Calves. Animals (Basel). 26;9(8):490. doi: 10.3390/ani9080490.

Dufreneix, F., Faverdin, P. and Peyraud, J.L. 2019. Influence of particle size and density on mean retention time in the rumen of dairy cows. Journal of Dairy Science,102(4): 3010-3022. https://doi.org/10.3168/jds.2018-15926.

Flemming, S., and L. A. H. Nielsen. 2001. An analysis on mortality among calves in Danish dairy herds. Page 79 in Proc. 11th Int. Conf. on Production Diseases of Farm Animals, Copenhagen, Denmark. Acta Veterinaria Scandinavica.

Fritz, J., Streich, W. J., Schwarm, A. and Clauss, M. 2012. Condensing results of wet sieving analyses into a single data: a comparison of methods for particle size description. Journal of Animal Physiology and Animal Nutrition. 96:783–797.

Fujikura, T., Oura, R. and Sekine, J. 1989. Comparative morphological studies on digestion physiology of herbivores. I. Digestibility and particle distribution of digesta and feces of domestic and feral animals. J. Fac. Agric. Tottori Univ. 25, 87-93.

Han, X., Dong, W. and Bao, J. 2021. Upgrading steam pretreatment by converting water-solublecarbohydrates into lactic acid prior to pretreatment. Biomass Conversion and Biorenfinery. https://doi.org/10.1007/s13399-020-01183-1.

Jamieson, W.S. and Hodgson, J. 1979. The effect of daily herbage allowance and sward characteristics upon the ingestive behaviour and herbage intake of calves under strip grazing management. Grass and Forage Science. 34:261-271.

Kamiya, M., Matsuzaki, M., Orito, H., Kamiya, Y., Nakamura, Y. N., and Tsuneishi, E. 2009. Effects of feeding level of milk replacer on body growth, plasma metabolite and insulin concentrations, and visceral organ growth of suckling calves. Animal Science Journal. 80: 662–668. doi: 10.1111/j.1740-0929.2009.00690.

Khan, M.A., Weary, D.M. and von Keyserlingk, M.A.G. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. Journal of Dairy Science. 94(7): 3547-3553.

Khanal, P.D., Axel, A.M., Safayi, S., Elbrønd, V.S. and Nielsen, M.O. 2020. Prenatal over- and undernutrition differentially program small intestinal growth, angiogenesis, absorptive capacity, and endocrine function in sheep. Physiol Rep. 8(2020): e14498. https://doi:10.14814/phy2.14498

Lauper, M., Lechner, I., Barboza, P.S., Collins, W.B., Hummel, J., Codron, D. and Clauss, M. 2013. Rumination of different-sized particles in muskoxen (Ovibos moschatus) and moose (Alces alces) on grass and browse diets, and implications for rumination in different ruminant feeding types, Mammalian Biology. 78(2): 142-152. https://doi.org/10.1016/j.mambio.2012.06.001.

Lechner-Doll, M., Kaske, M. and Von Engel-hardt, W. 1991. Factors affecting the mean retention time of particles in the forestomach of ruminants and camelids. In: T. Tsuda, Y. Sasaki, R. Kawashima (eds), Physiological Aspects of Digestion and Metabolism in Ruminants. Academic Press, San Diego, pp. 445-482.

Long, N. M., Vonnahme, K. A., Hess, B. W., Nathanielsz, P. W. and Ford, S. P. 2009. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. Journal of Animal Science, 87:1950-1959. https://doi.org/10.2527/jas.2008-1672.

Lyford, S. J. Jr. 1988. Growth and development of the ruminant digestive system. Pages 44-63 in The Ruminant Animal: Digestive Physiology and Nutrition. D. C. Church, ed. Prentice Hall, Englewood Cliffs, NJ.

McGovern, F.M., Campion, F.P., Sweeney, T., Fair, S., Lott, S. and Boland, T.M. 2015. Altering ewe nutrition in late gestation: II. The impact on fetal development and offspring performance. Journal of Animal Science, 93:4873–4882 doi:10.2527/jas2015-9020

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

Modina, S.C., Aidos, L., Rossi, R., Pocar, P., Corino, C. and Di Giancamillo, A. 2021. Stages of Gut Development as a Useful Tool to Prevent Gut Alterations in Piglets. Animals, 11, 1412. https://doi.org/10.3390/ani11051412.

Nascimento, K.B., Galvão, M.C., Meneses, J.A.M., Moreira, G.M., Ramírez-Zamudio, G.D., Souza, S.P.d., Prezotto, L.D., Chalfun, L.H.L., Duarte, M.d.S., Casagrande, D.R. and Gionbelli, M.P. 2022. Effects of Maternal Protein Supplementation at Mid-Gestation of Cows on Intake, Digestibility, and Feeding Behavior of the Offspring. Animals 2022, 12, 2865. https:// doi.org/10.3390/ani12202865.

Paradis, F., Wood, K., Swanson, K.C., Miller, S.P., McBride, B.W. and Fitzsimmons, C. 2017. Maternal nutrient restriction in mid-to-late gestation influences fetal mRNA expression in muscle tissues in beef cattle. BMC Genomics, 18:632. https://doi.org/10.1186/s12864-017-4051-5.

Penzo-Mendez, A. I., and Stanger, B. Z. 2015. Organ-size regulation in mammals. Cold Springs Harb. Perspect. Biol. 7, 1–11. doi: 10.1101/cshperspect.a019240.

R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020.

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

Reynolds, L. P., Borowicz, P. P., Caton, J. S., Crouse, M. S., Dahlen, C. R., and Ward, A. K. 2019. Developmental programming of fetal growth and development. Vet. Clin. NA Food Anim. Pract. 35, 229–247. doi: 10.1016/j.cvfa.2019.02.006.

Seo, S., Lanzas, C., Tedeschi, L.O., Pell, A.N and Fox, D. G. 2009. Development of a mechanistic model to represent the dynamics of particle flow out of the rumen and to predict rate of passage of forage particles in dairy cattle. Journal of Dairy Science. 92: 3981–4000

Swartz, T.H. and Petersson-Wolfe, C.S. 2022. Associations between preweaning calf feeding behaviors with age at first calving and lactational performance using an automatic calf feeder. JDS Communications, https://doi.org/10.3168/jdsc.2022-0255.

Tao, S., Dahl, G. E., Laporta, J., Bernard, J. K., Orellana Rivas, R. M., and Marins, T. N. 2019. Physiology symposium: effects of heat stress during late gestation on the dam and its calf. Journal of Animal Science. 97:2245–2257. doi: 10.1093/jas/skz061.

Thornbury, J. C., Sibbons, P. D., van Velzen, D., Trickey, R. and Spitz, L. 1993. Histological investigations into the relationship between low birth weight and spontaneous bowel damage in the neonatal piglet. Pediatr. Pathol. 13: 59.

Trahair, J.F., DeBarro, T.M., Robinson, J.S. and Owens, J.A. 1997. Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. Journal of Nutrition.127(4):637-41. doi: 10.1093/jn/127.4.637. PMID: 9109616.

Trotta, R.J, Vasquez-Hidalgo, M.A, Vonnahme, K.A and Swanson, K.C. 2020. Effects of Nutrient Restriction During Midgestation to Late Gestation on Maternal and Fetal Postruminal Carbohydrase Activities in Sheep. J Anim Sci. 98(1): skz393. doi: 10.1093/jas/skz393.

Trotta, R.J. and Swanson, K.C. 2021. Prenatal and Postnatal Nutrition Influence Pancreatic and Intestinal Carbohydrase Activities of Ruminants. Animals, 11: 171.

Uden, P. and Van Soest, P.J. 1982. The determination of digesta particle size in some herbivores. Animal Feed Science and Technology. 7:35-44.

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

Wathes, D.C. 2022. Developmental Programming of Fertility in Cattle—Is It a Cause for Concern? Animals, 12: 2654. https://doi.org/10.3390/ ani12192654.

Xu, R.J., Mellor, D. J., Birtles, M. J., Reynolds, G. W. and Simpson, H. V. 1994. Impact of intrauterine growth retardation on the gastrointestinal tract and pancreas in newborn pigs. J. Pediatr. Gastroenterol. Nutr. 18: 231.

Zhang Y., Otomaru K., Oshima K., Goto Y., Oshima I., Muroya S., Sano M., Saneshima R., Nagao Y., Kinoshita A., Okamura, Y., Roh, S., Ohtsuka, A. and Gotoh, T. 2021. Effects of low and high levels of maternal nutrition consumed for the entirety of gestation on the development of muscle, adipose tissue, bone, and the organs of Wagyu cattle fetuses. Animal Science Journal. 92: e13600. doi: 10.1111/asj.13600.

APPENDIX 2

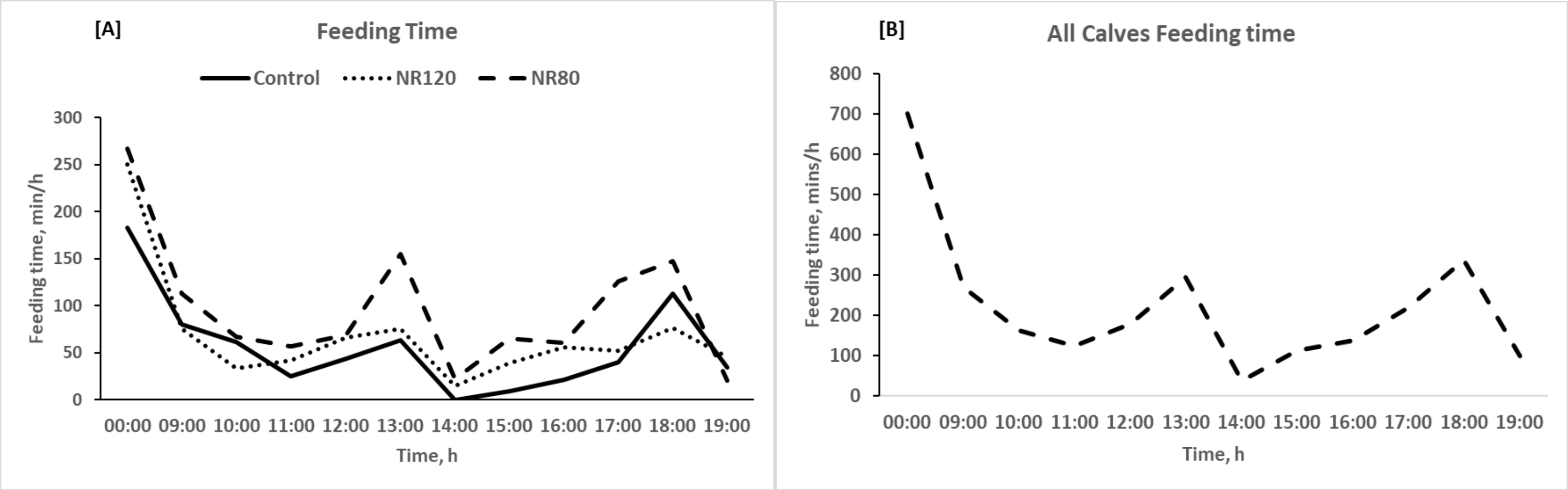


Figure 5.8. a-b. Hourly variation of feeding pattern in post weaned calves born to mothers fed either: NR80=0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120=0.6 M until day 120 of gestation and Control=ad libitum (1.8 M) until day 120 of gestation

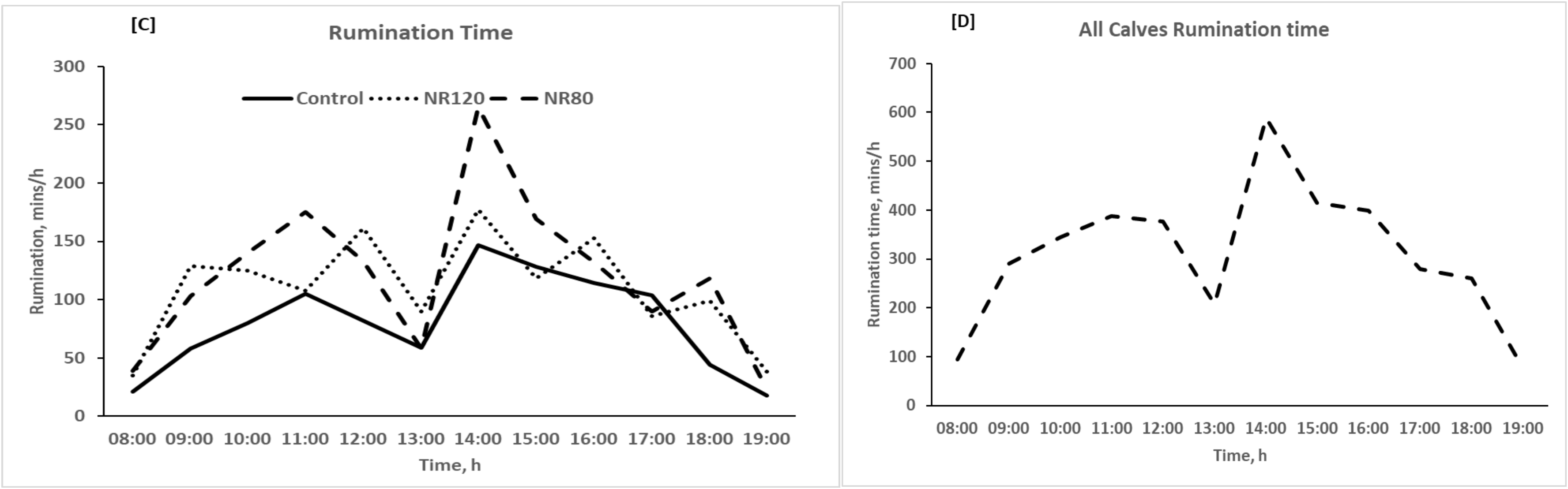


Figure 5.8. c-d. Hourly variation of rumination pattern in post weaned calves born to mothers fed either: NR80=0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120=0.6 M until day 120 of gestation and Control=ad libitum (1.8 M) until day 120 of gestation

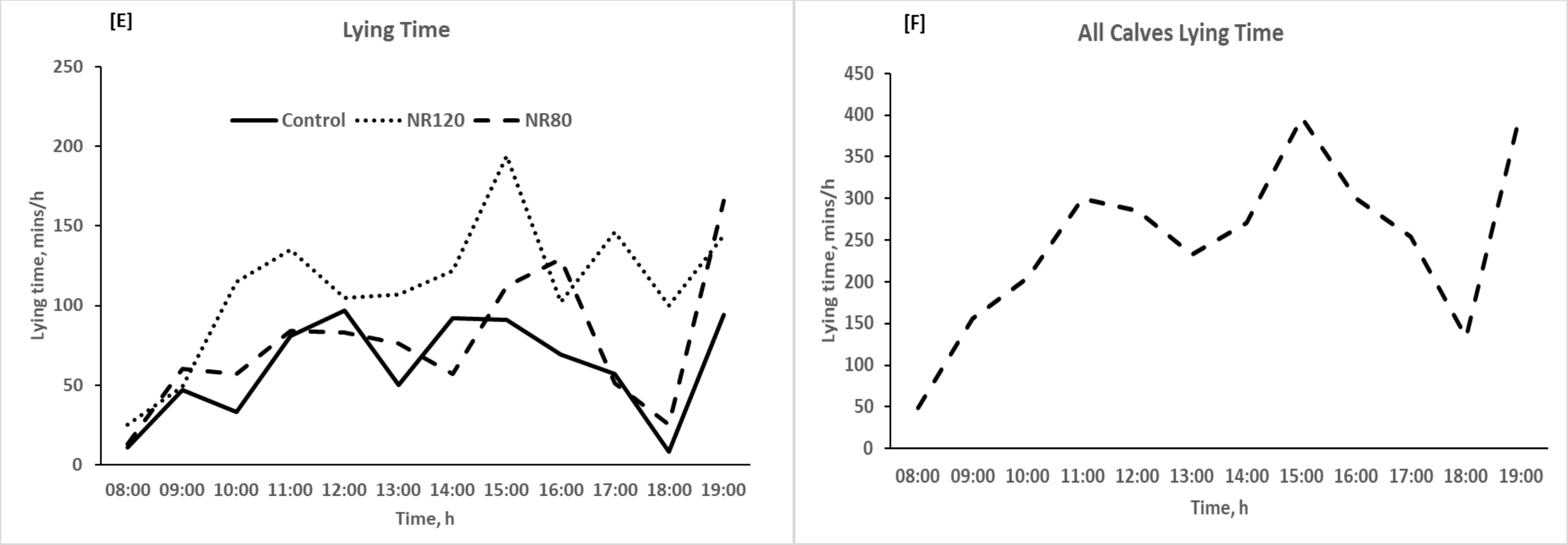


Figure 5.8. e-f. Hourly variation of resting pattern in post weaned calves born to mothers fed either: NR80=0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120=0.6 M until day 120 of gestation and Control=ad libitum (1.8 M) until day 120 of gestation

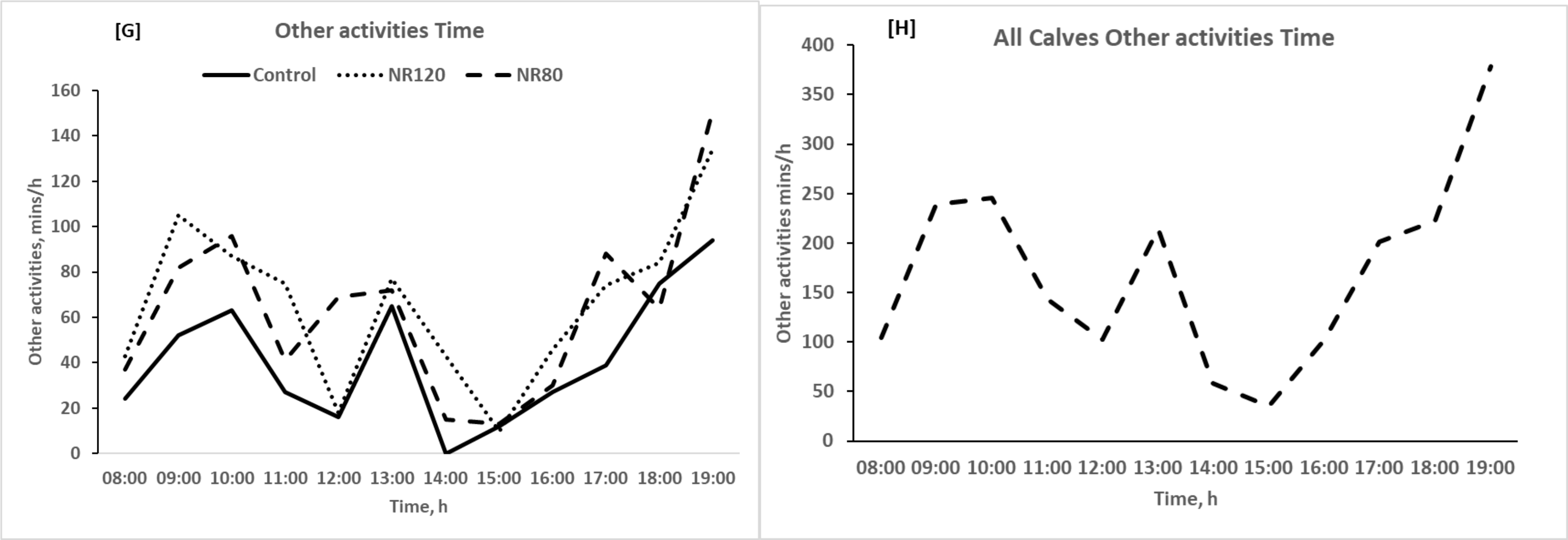


Figure 5.8. g-h. Hourly variation of other activities in post weaned calves born to mothers fed either: NR80=0.6 M of their maintenance energy requirements (M) until day 80 of gestation NR120=0.6 M until day 120 of gestation and Control=ad libitum (1.8 M) until day 120 of gestation.

GENERAL CONCLUSIONS

Based on the results obtained from this study, it allows to conclude as follows:

Gestating heifers

- The concept that maternal energy requirement in heifers during early gestation is of less importance is misleading as compelling evidence from the current study show marked decrease in growth performance and welfare concerns during period of restriction.
- Maternal nutrition starting 11 days prior to artificial insemination did not negatively influence pregnancy rate and was highly comparable to average targeted pregnancy rate of >55% for first service heifers. This set the stage study developmental programming throughout pregnancy.
- Maternal growth performance in heifers during gestation is inherently dependent on the nutritional status of the animals from early gestation.
- When pregnant heifers are exposed to re-alimentation, after a period of restriction, there is marked catch-up growth that supports body tissue growth and skeletal frame development needed to calve.
- Dry matter intake, by design, was higher in control fed heifers than nutrient restricted fed heifers in early gestation. However, when DMI was evaluated based on model equations, this ascendency persisted, suggesting that DMI throughout pregnancy also depends on the nutritional status of the animals from early gestation.
- Remarkably, DMI in nutrient restricted heifers declined in the last days to calving while that of control fed heifers remained similar.
- Behavioural activities, especially rumination patterns, are markedly reduced when limit feeding is initiated. Limiting feed intake during pregnancy results in heifers ruminating

less than 300mins/day, a threshold indicative of good gut health. However, there is a tremendous improvement in rumination activity when pregnant heifers are exposed to refeeding, suggesting that there is no carryover negative implication of restricted feeding during early gestation.

- Gestating NR heifers had better crude protein total tract digestibility compared to control fed heifers at d 70 of pregnancy. We speculate that this greater CP digestibility in NR pregnant heifers may explain why the body weight remained constant throughout the differential feeding period. However, as gestation progresses, the apparent nutrient digestibility of nutrients decreased across all groups.
- Prenatal nutrition in early gestation did not affect the gestational length of heifers neither did it perturbed calving difficulties.
- Altering maternal energy requirement in early gestation reduced body conditions of heifers throughout pregnancy. However, at calving restricted heifers showed equal BCS compared to their peers.
- Regardless of feeding program to which heifers were exposed, BCS in all heifers reduced after calving, suggesting adequate mobilization of fat stored in the adipose tissue, promoting the fatty acid metabolic pathway to increase milk fat.
- Heifers in the NR80 group did not exhibit positive adaptive responses when transitioning from a nutrient-restricted environment to ad libitum access, as evidenced from the phenotypic description at birth and duodenum development in their offspring.

Daughters (Offspring)

- Daughter calves born to overfed mothers are heavier (~4.7kg heavier) at birth compared to daughters from nutrient restricted mothers. Furthermore, they had larger thoracic circumference and tend to be taller than their counterparts. However, as calves age, this ascendancy disappeared, thus suggesting that calves have equal chance to grow and show capacity of catch-up growth.
- Restricting maternal energy requirement from preconception to early gestation produces calves with higher total solid feed intake than control calves. However, despite this increase in solid feed intake, all body measurements and apparent nutrient digestibility estimates were similar among calves. Positively, higher solid intake by NR80 calves, resulted in higher rumination time in weeks 3 and 4 preweaning.
- Perturbations in early gestation maternal energy intake did not affect DMI, body weight, BCS, and other body measurements in calves at weaning.
- Altering maternal energy requirement during early pregnancy had no effect on postweaning DMI, growth performance, or apparent total tract digestibility in calves. However, NR80 calves continued to exhibit dominant behavioural eating and ruminating patterns, implying that these calves were programmed to compensate for their low birth weight by displaying these hierarchical behavioural patterns between pre- and post-weaning.
- Early gestation maternal energy restriction or overfeeding of heifers did not alter slaughter performance in post weaned calves. The weight of the foregut was similar among all calves. However, control calves tended to have higher duodenal weights than

daughters from nutrient restricted mothers, suggesting impaired development of the duodenum in calves born to nutrient restricted mothers

- Regardless of prenatal nutrition, dry matter distribution of digesta along the calves GIT was uneven with higher dry matter concentration detected in the foregut (especially the rumen) than the lower gut. Similarly, mean particle size (dMEAN) was higher in the foregut than the lower gut.
- Using sieve fractions, proportions of digesta particles retained on the different sieves markedly differed from those ingested. Higher digesta particles were retained in the smaller sieves than ingested particles. Further, finer digesta were obtained in the lower gut than the upper gut. This result indicates normal digestive physiology of the system.
- This study was subject to limitations. In order to assess the immediate impact of maternal diet on *in utero* organ development, some calves may be slaughtered at birth or during weaning if several heifers are recruited. This is inspired from several studies particularly those on heat stress, that showed calves that were euthanized at birth or at weaning had lower weights of important visceral organs. Additionally, the nutritional status of the dams, dating back to their preweaning and weaning periods, is crucial information in establishing if the feeding program (early gestation undernutrition or overnutrition) used in this study is sufficient to cause postnatal disturbances in the offspring.
- Finally, this study adds to the growing body of evidence that maternal nutrient restriction in dairy heifers during early gestation has the capacity to reprogram phenotypic traits in their offspring at birth with a potential to impair the development of the gastrointestinal tract at 135 days of postnatal life.

IMPLICATIONS AND FUTURE CONSIDERATIONS

Scientific implication

Despite the fact that it is still unclear how placental and fetal programming work, the "fetal or developmental programming" theory of cow-calf nutrition has significant consequences for the cattle industry and demands the attention of farmers, producers, and researchers. The results of the current study have a number of potential applications for cow-calf rearing programs.

Data from the current study contradicts historic assumptions and show that limit fed heifers, starting 11 days before artificial insemination and throughout early pregnancy markedly loose body weight and body condition and suggesting that the animals growth performance throughout gestation is dependent on their nutritional status in early gestation. Consequently, calves born from nutrient restricted mothers were lighter at birth and showed a tendency for impaired duodenum development. This suggest that poor nutrition of mothers in early gestation does not only have the potential to limit the dam's growth performance during important cellular differentiation activities at embryonic stage but may potentially impair body weight at birth and important GIT organs.

Inspired by previous studies in humans (Barker hypothesis from the Dutch famine), and animal models, birth weight is an indicator of the production performance of the individual later in life. With the low birth weights in calves born to nutrient restricted mothers, diary scientists researching fetal programming need to rethink models to include birth weights predictors despite evidenced catch up growth after realimentation.

Regardless of maternal nutrition, calves from this study seem to show a better transition from liquid to solid feed by not only increasing their daily feed intake, but also by altering feeding patterns. Limiting milk replacer intake helps to promote rumen development by accelerated starter intake prior to weaning. However, the increased starter intake did not alter apparent nutrient digestibility and growth rate as calves grew uniformly, neither did it trigger a difference in rumen development between control calves and their peers. This information paves the way for future research on the interaction between compensatory growth during gestation and offspring solid intake during preweaning because increased feed intake in NR80 calves during preweaning, did not improve growth rates and digestibility coefficients throughout their postnatal life.

Interestingly, the results of this study have consistently demonstrated that calves develop catch up abilities as the age. It is anticipated that this growth trajectory will increase the survival and longevity of calves in the herd. What remains unclear is if this “catch up” capacity persist throughout their lifetime.

Findings from this thesis further show that despite previous beliefs that refeeding after periods of feed restriction during pregnancy would compensate for underdeveloped *in utero* visceral organs in calves, results show that the duodenum, which is the first part of the small intestine where absorption of nutrients begins, could be impaired in calves born to nutrient restricted mothers despite long periods of realimentation. Assuming that the impaired development of the duodenum continues into adulthood, the organ might be less able to secrete the small intestine-specific enzymes (carbohydrase and glycohydrolase) needed to break down the glycosidic bond of saccharides to enhance nutrient digestibility and absorption in the lower gut.

Farm level

The consequences of our study at farm level raise concerns regarding animal welfare and the potential for compromised animal health. During differential feeding in the current study, heifers vocalized more and stood for longer periods of time. Additionally, they showed unpleasant behaviour of searching for undigested feed in their waste.

A farm strategy that involves two or three steps to balance out positive and negative energy balances may be harmful to the overall survival and productivity of the offspring, especially given the level of maternal nutrition that was imposed on the heifers in the current study. Early postnatal life losses, particularly from the digestive and immune systems, might be made worse by organ juvenile stage and IUGR-related failure. Because the main immunoprotective mechanism in the bovine is passive uptake of immunoglobulins, new-born calves are consequently extremely vulnerable to insults that could reduce the transfer of immunoglobulins in colostrum.

Because the processes of organ formation start early in pregnancy and continue throughout gestation, if farmers choose to adopt a two-step approach that restricts feeding during preconception and the first few months of pregnancy (perhaps to save feed cost), followed by *ad libitum* access to nutrients starting at mid gestation and continuing until calving (demonstrated in the current study), fetal organs may be at risk of maldevelopment. The purpose of the dairy industry is to raise animals that can produce milk in a sustainable way. Consequently, for instance, ensuring that the mammary gland grows in an isometric manner throughout gestation should be the focus of dairy farmers. Likewise, since milk production and nutrient digestion and utilization works in a dependent like manner, it is imperative that dairy farmers rethink heifer nutrition throughout pregnancy.

Presently, prenatal care is transitioning to incorporate goals of optimizing maternal, fetal, and neonatal health to prevent or reduce adult-onset diseases. Guidelines regarding optimal pregnancy nutrition and weight gain, management of low- and high-fetal-weight pregnancies, use of maternal glucocorticoids, and new-born feeding strategies, among others, have yet to fully integrate long-term consequences on adult health. Knowledge of cattle fetal programming can help the cattle industry rethink cattle nutrition differently.

Future research on fetal programming in cattle is anticipated to bring together the efforts of nutritionists and geneticists in order to identify the genes that are most affected by fetal programming, establish their effects, and develop therapies to undo DNA methylation caused by nutritional deficiencies in cattle. Therefore, it is expected that future study into cow fetal programming will continue.

Given the relationship between fetal programming and maternal nutrition, and the link between academia and livestock feed industries, it is crucial that any novel, low-cost feeds that are introduced into the market be carefully assessed for their impact on fetal development. Additionally, a close monitoring of generations should be encouraged as the developmental origin of health and diseases (DoHAD) should form the bedrock for selection of replacement heifers.