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Effects of maternal intake of metabolizable energy during the late gestation on the energy metabolism of skeletal muscle in beef offspring

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Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

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“The things we truly love stay with us always, locked in our hearts as long as life remains”.
(Josephine Baker)

ABSTRACT

KLADT, Luiza Vitarelli, M.Sc., Universidade Federal de Viçosa, July, 2025. **Effects of maternal intake of metabolizable energy during the late gestation on the energy metabolism of skeletal muscle in beef offspring**. Adviser: Cristina Mattos Veloso. Co-adviser: Marcio de Souza Duarte.

The cow-calf phase is considered the foundation of beef cattle production, playing a pivotal role in the overall performance and efficiency of the system. While genetic traits and postnatal management strategies are relevant, there is growing recognition that maternal nutrition during gestation plays a critical role in programming fetal development. In particular, the final third of gestation is a crucial period characterized by rapid fetal growth and a marked increase in the cow's nutritional requirements. In beef production systems, both in tropical and temperate regions, this period often coincides with nutritional constraints related to seasonal pasture unavailability or greater reliance on economically costly diets. Changes in maternal energy intake during this stage may trigger significant metabolic adaptations in the fetus, potentially influencing skeletal muscle metabolism and feed efficiency in the offspring throughout its productive life. In this context, this dissertation investigated the effects of different levels of maternal metabolizable energy intake during the last third of gestation on the skeletal muscle energy metabolism of beef calves. Pregnant Angus-Simmental (n = 42) cows were fed diets providing 92%, 104%, or 118% of the estimated energy requirements for this period. No significant differences were observed in calf performance among treatments, including birth weight ($P > 0.05$), weaning weight ($P > 0.05$), average daily gain ($P > 0.05$), or blood metabolic profile (insulin, glucose, beta-hydroxybutyrate, and non-esterified fatty acids; $P > 0.05$). However, calves from cows fed 92% of energy requirements showed higher AMPK activity ($P < 0.01$), along with greater expression of the genes PPARa ($P = 0.04$), PPARGC1a ($P = 0.04$), and MEF2A ($P = 0.01$). Expression of MYH2a and MYH2x genes was also higher in this group ($P = 0.04$ and $P = 0.01$, respectively). Akt activity was greater in both the 92% and 118% groups compared to the control group ($P = 0.01$). These results indicate that maternal nutrition in late gestation can modulate offspring muscle metabolism, influencing mitochondrial biogenesis, metabolic flexibility, and potentially the efficiency of nutrient utilization in later phases of beef production.

Keywords: energy metabolism; fetal programming; maternal nutrition; metabolic flexibility; metabolizable energy; skeletal muscle

RESUMO

KLADT, Luiza Vitarelli, M.Sc., Universidade Federal de Viçosa, julho de 2025. **Efeitos da ingestão materna de energia metabolizável durante o terço final da gestação sobre o metabolismo energético do músculo esquelético da progênie bovina**. Orientadora: Cristina Mattos Veloso. Coorientador: Marcio de Souza Duarte.

A fase de cria é considerada o alicerce da pecuária de corte, sendo determinante para o desempenho e a eficiência do sistema como um todo. Embora características genéticas e estratégias de manejo pós-natal sejam relevantes, há crescente reconhecimento de que a nutrição materna durante a gestação desempenha papel essencial na programação do desenvolvimento fetal. Em especial, o terço final da gestação representa um período crítico, caracterizado por rápido crescimento fetal e aumento expressivo das exigências nutricionais da vaca. Em sistemas de produção de bovinos de corte, tanto em regiões tropicais quanto temperadas, esse período pode coincidir com restrições nutricionais relacionadas à sazonalidade das pastagens ou à maior dependência de dietas que são economicamente mais onerosas. Alterações na ingestão de energia pela vaca durante essa fase podem desencadear adaptações metabólicas importantes no feto, com possíveis reflexos no metabolismo muscular e na eficiência alimentar da progênie ao longo da vida produtiva. Neste contexto, esta dissertação investigou os efeitos de diferentes níveis de ingestão materna de energia metabolizável durante o terço final da gestação sobre o metabolismo energético do músculo esquelético de bezerros de corte. Vacas prenhes da raça Angus-Simental (n = 42) foram alimentadas com dietas fornecendo 92%, 104% ou 118% das exigências energéticas estimadas para esse período. Não foram observadas diferenças significativas no desempenho dos bezerros entre os tratamentos, incluindo peso ao nascimento ($P > 0,05$), peso ao desmame ($P > 0,05$), ganho médio diário ($P > 0,05$) e perfil metabólico sanguíneo (insulina, glicose, beta-hidroxibutirato e ácidos graxos não esterificados; $P > 0,05$). No entanto, bezerros provenientes de vacas alimentadas com 92% das exigências apresentaram maior atividade da AMPK ($P < 0,01$), além de maior expressão dos genes PPARa ($P = 0.04$), PPARGC1a ($P = 0.04$) e MEF2A ($P = 0.01$). A expressão dos genes MYH2a e MYH2x também foi maior nesse grupo ($P = 0,04$ e $P = 0,01$, respectivamente). A atividade da Akt foi superior nos grupos alimentados com 92% e 118% das exigências em comparação ao grupo controle ($P = 0,01$). Esses resultados indicam que a nutrição materna no final da gestação é capaz de modular o metabolismo muscular da progênie, influenciando a biogênese mitocondrial, a flexibilidade metabólica e, possivelmente, a eficiência no

uso de nutrientes em fases posteriores da pecuária de corte.

Palavras-chave: energia metabolizável; flexibilidade metabólica; metabolismo energético; músculo esquelético; nutrição materna; programação fetal

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SUMMARY

GENERAL INTRODUCTION

The cow-calf phase is a foundation stage in beef production, directly influencing the productivity and profitability of the entire beef supply chain. Success in this phase is closely tied to both maternal and offspring performance (Diskin & Kenny, 2016; Sanz et al., 2024). Cow-calf operations aim to produce healthy, fast-growing calves that will ultimately become efficient feedlot animals and yield high-quality carcasses (Danyer et al., 2024). However, the productivity of this phase is not solely determined by genetics or postnatal management, but also by the maternal environment experienced during gestation (Costa et al., 2021a; Moriel et al., 2021).

Across both temperate and tropical systems, the final third of gestation often coincides with seasonal constraints that can compromise maternal nutrition. In temperate systems, the defined breeding season results in late gestation occurring during winter, when pasture is unavailable. As a consequence, the nutritional requirements of the pregnant cow must be met through the provision of stored forages and feeds (Pearson et al., 2019). Conversely, in tropical and subtropical regions, although cows are primarily managed on pasture, the dry season imposes a sharp decline in forage availability and nutritional quality during late gestation (Santos et al., 2022). In both contexts, pregnant cows may be exposed to varying degrees of negative energy balance, precisely when the rate of fetal growth and associated nutrient demands accelerate dramatically (Bell, 1995).

During late gestation, the exponential growth of the fetus significantly elevates the cow's energy requirements. If these demands are not met through adequate dietary intake, negative energy balance ensues, triggering a cascade of metabolic adaptations. In response, cows mobilize body fat reserves to compensate for the energy deficit, leading to elevated blood concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA), alongside reduced glucose concentrations (Grummer, 1995; Butler, 2000). These metabolic adaptations, although physiologically necessary, can compromise maternal homeostasis and increase the risk of peripartum disorders (Drackley, 1999). The degree of negative energy balance is strongly influenced by maternal diet, particularly the energy density and availability of nutrients during late gestation. Importantly, the consequences of negative energy balance are not limited to maternal performance but may also extend to the developing fetus, potentially compromising offspring growth, metabolic development, and long-term productivity (Funston et al., 2010; Klein et al., 2021; Waldon et al., 2023). These interconnected effects highlight the need to better understand how maternal nutrition during this critical period can influence not

only the dam's physiological status but also the efficiency and sustainability of the entire cow-calf production system.

In several studies, protein or energy supplementation was linked to greater maternal performance (Costa et al., 2022; Lopes et al., 2020; Meneses et al., 2024; Wilson et al., 2015). In a pasture-based study, Lopes et al. (2020) demonstrated that protein supplementation in cows grazing low-quality forage reduced maternal tissue mobilization, indicating improved metabolic balance. Similar outcomes were observed by Costa et al. (2022), with rumen-undegradable protein supplementation during late gestation leading to greater maternal body weight at calving. However, maternal supplementation does not always result in measurable performance benefits. In the study of Hare et al. (2019), feeding cows 133% of their metabolizable protein requirements did not significantly affect maternal body weight, body condition score (BCS), or other physiological parameters. Likewise, Wilson et al. (2015) reported that increased maternal energy intake altered body weight and BCS, but had no significant impact on milk production or subsequent pregnancy rates. Conversely, nutrient restriction during late gestation has been associated with marked changes in maternal metabolism and performance (Waldon et al., 2023). Restricted cows often exhibit lower circulating levels of glucose, urea nitrogen, and triglycerides, alongside elevated concentrations of non-esterified fatty acids and cortisol (Redifer et al., 2023). Beyond these maternal outcomes, extensive research has focused on how gestational nutrition modulates the cellular composition of fetal tissues, particularly the formation and development of skeletal muscle (Du 2023).

Fetal programming, also referred to as developmental programming, describes the process by which environmental stimuli during critical periods of gestation induce lasting changes in the structure, physiology, and metabolism of the offspring. This concept, initially proposed through epidemiological observations in humans, has since been widely demonstrated in animal models (Baker et al., 1989; Baker, 1993; Wu et al., 2006). In the context of livestock production, fetal programming has received increasing attention due to its implications for growth efficiency, carcass and meat quality, and overall animal performance (Du et al., 2010). During gestation, particularly in late pregnancy, when fetus undergoes rapid development, disruptions in maternal nutrition can do permanent modifications in offspring tissues and metabolism. These include changes in the balance of cell types formed, the composition of muscle and adipose tissues, and metabolic pathways that persist into postnatal life (Barcelos et al., 2022; Costa et al., 2021a; Du, 2023; Santos et al., 2022). Consequently, maternal metabolism during gestation should be considered as a relevant background factor influencing fetal responses to nutritional inputs.

Maternal energy and protein interventions during late gestation can lead to variable outcomes in beef offspring, depending on the type and magnitude of the intervention, as well as maternal and environmental conditions (Shokrollahi et al., 2025). Some studies have reported positive effects of maternal supplementation, such as increased birth weight (Wilson et al., 2016) or greater weaning weight (Stalker et al., 2006). Conversely, some studies assessing maternal supplementation strategies during gestation reported no significant effects on offspring performance (Marquez et al., 2017; Sanglard et al., 2022). Similar inconsistencies have been observed under maternal nutrient restriction during late gestation, with some reports indicating reduced birth weight (Klein et al., 2022), while others found no effect on postnatal growth or weight gain in the progeny (Maresca et al., 2019).

Despite the variable effects of maternal supplementation on offspring performance traits, molecular evidence suggests that late-gestation nutritional interventions can influence the cellular development of skeletal muscle. In a study by Costa et al. (2022), maternal supplementation during the final third of gestation resulted in increased expression of *PDGFRα*, *ZFP423*, *PPARγ*, and *C/EBPα* in the offspring's skeletal muscle, along with greater abundance of *ZFP423* and *PPARγ* at the protein level. These changes indicate an enhanced adipogenic commitment within muscle tissue. Similar outcomes were observed by Duarte et al. (2014), who reported that maternal overnutrition during late gestation led to greater expression of the same adipogenic markers and was associated with increased collagen deposition in fetal skeletal muscle.

While maternal overnutrition during late gestation has been associated with enhanced adipogenic programming in fetal muscle, nutrient restriction during the same period can also induce distinct adaptations with potential consequences for muscle development and metabolic function. Paradis et al. (2017) evaluated the effects of maternal energy restriction during mid-to-late gestation in beef cattle and reported altered expression of genes involved in muscle growth, myogenesis, and adipogenesis in fetal skeletal muscle, despite the absence of phenotypic differences in fetal weight or size. Notably, restricted fetuses exhibited increased mRNA levels of *IGF1*, *IGF1R*, *IGF2R*, *INSR*, *MYOD1*, *MYOG*, and *PPARγ*, suggesting a compensatory response aimed at preserving muscle development under limited nutrient availability. Complementary findings were reported by Costa et al. (2021b), who observed transcriptomic changes in the skeletal muscle of newborn goats following maternal feed restriction during late gestation. Offspring from restricted dams showed upregulation of genes associated with oxidative metabolism, oxidative stress, and impaired insulin sensitivity. However, the data also indicated activation of protective mechanisms, likely aimed at

maintaining cellular homeostasis under prenatal nutritional stress. Together, these results suggest that maternal nutrient restriction in late gestation may not always manifest in immediate phenotypic deficits but can induce molecular signatures in fetal muscle related to altered energy metabolism, insulin signaling, and long-term metabolic flexibility.

Skeletal muscle is a metabolically dynamic tissue that exhibits plasticity, defined as its capacity to undergo structural and metabolic adaptations in response to environmental stimuli, including nutrient availability and hormonal signals (Talbot & Maves, 2016; Schiaffino et al., 2013). This plasticity is essential for the regulation of metabolic flexibility, which refers to the muscle's ability to shift between glucose and fatty acid oxidation depending on physiological conditions (Shoemaker et al., 2023). The regulation of metabolic flexibility in skeletal muscle involves several nutrient-sensitive signaling pathways, including insulin–IRS1-Akt and AMP-activated protein kinase (AMPK). These pathways collectively govern mitochondrial biogenesis, oxidative metabolism, and substrate preference (Lin et al., 2002; Liang and Ward, 2006). When the ability to switch between energy substrates is impaired, a condition known as metabolic inflexibility can arise, which has been associated with reduced insulin sensitivity, altered mitochondrial function, and an increased risk of metabolic dysfunction later in life (Shoemaker et al., 2023).

The insulin–IRS1–Akt pathway is fundamental for regulating skeletal muscle metabolism, integrating signals from nutrients, hormones, and energy status to control glucose uptake and growth (Long et al., 2011). Upon insulin stimulation, IRS1 activates PI3K, leading to Akt phosphorylation, which in turn promotes glucose transporter translocation to the plasma membrane and enhances glucose uptake in muscle fibers (Saltiel & Kahn, 2001). This pathway also supports anabolic processes such as muscle growth, glycogen synthesis, and lipid metabolism, and its disruption leads to impaired muscle development, increased catabolic signaling, and insulin resistance (Pessin & Saltiel, 2000). AMP-activated protein kinase (AMPK) is a central energy sensor in skeletal muscle, activating catabolic pathways to restore ATP during metabolic stress and promoting glucose uptake and fatty acid oxidation while inhibiting energy-consuming processes (Wang et al., 2012). AMPK exerts longer-term effects by directly phosphorylating and activating *PPARGC-1α*, a master regulator of mitochondrial biogenesis and oxidative metabolism, and also indirectly through SIRT1-mediated deacetylation (Cantó et al., 2009). This AMPK–*PPARGC-1α* axis upregulates genes involved in mitochondrial function, fatty acid oxidation, and the formation of oxidative (type I) muscle fibers, thereby enhancing metabolic flexibility (Kong et al., 2022). *PPARGC-1α* further coactivates nuclear receptors such as *PPARα*, which control the expression of genes for fatty

acid transport and oxidation, amplifying the muscle's oxidative capacity (Burri et al., 2010). Disruption of these pathways, as seen in insulin resistance or during adverse fetal development, impairs mitochondrial function and muscle metabolism, leading to reduced insulin sensitivity and compromised energy homeostasis (Hardie et al., 2012).

Recent animal studies provide evidences that maternal nutrition during gestation can alter skeletal muscle energy metabolism in the offspring, modulating genes involved in insulin signaling, oxidative metabolism and mitochondrial function. In beef calves, Sanglard et al. (2018) observed that energy restriction during late gestation led to changes in the muscle transcriptome related to lipid metabolism, mitochondrial activity, and energy metabolism. These transcriptional adaptations suggest a shift in metabolic programming that may impair postnatal flexibility in fuel utilization. Similarly, in a rodent study, Aragão et al. (2014) found that maternal protein restriction increased the expression of oxidative metabolism key regulatory genes and proteins, in the skeletal muscle of adult offspring maintained under ad libitum feeding. While this suggests an upregulated oxidative profile at baseline, the study also noted a reduced ability to adjust fuel usage in response to metabolic challenges, indicating impaired metabolic flexibility.

In contrast, maternal overnutrition has also been associated with disruptions in skeletal muscle metabolism. In cattle, Shokrollahi et al. (2024) observed that calves born to overfed cows exhibited increased expression of adipogenic genes (*ZFP423*, *C/EBPα*) and reduced expression of oxidative markers (*PPARGC1A*) in neonatal muscle. Supporting these findings, in two studies Zou et al. (2016; 2017) demonstrated in swine that maternal high-energy diets reduced fetal skeletal muscle differentiation and mitochondrial biogenesis. Together, these results suggest that both nutritional excess and deficiency during late gestation can alter the metabolic programming of skeletal muscle, these disruptions may compromise the offspring's capacity to adapt fuel utilization across different physiological conditions, with potential long-term consequences for growth efficiency and metabolic health.

Given the consistent evidence that both maternal energy restriction and overnutrition during late gestation can alter skeletal muscle development and metabolism in the offspring, there is a clear need to characterize how specific levels of maternal metabolizable energy intake affect these outcomes. Therefore, the present study aimed to investigate the effects of different maternal metabolizable energy levels during late gestation on the offspring of beef cows, with a specific focus on energy metabolism in skeletal muscle. By evaluating molecular and metabolic parameters, this research seeks to provide novel insights into the mechanisms by which maternal nutrition programs muscle function.

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MATERNAL METABOLIZABLE ENERGY INTAKE DURING LATE GESTATION AFFECT ENERGY METABOLISM OF THE SKELETAL MUSCLE OF BEEF OFFSPRING

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ABSTRACT

The objective of this study was to evaluate the effects of varying levels of maternal metabolizable energy (ME) intake during late gestation on the changes in postnatal development of skeletal muscle in calves. A total of 42 primiparous (n = 21) and multiparous (n = 21) pregnant Angus-Simmental beef cows (680.8 ± 74.4 kg) were housed indoors at the Ontario Beef Research Center (OBRC) at the University of Guelph. Cows were blocked by predicted calving date, balanced by initial body weight and parity, and randomly assigned to one of three treatment diets designed to provide 92% (LME, n = 16), 104% (CME, n = 13), or 118% (HME, n = 13) of predicted ME requirements, based on a 715 kg Angus cow with BCS 7 programmed to lose 2 BCS units over late gestation. All cows were managed under a planned moderate negative energy balance starting 53 days before expected calving. At birth, calves were weighed before suckling the dam, and again day 209 at weaning. At 28 days of age, plasma and serum samples were collected for insulin, glucose, BHBA and NEFA analysis. At 30 days of age Longissimus muscle samples were biopsied from the calves and were used for mRNA expression and protein abundance for energy metabolism. All statistical analyses were performed in SAS Studio, in a mixed model including the fixed effects of treatment and parity, and the random effect of sire. No differences were observed among treatments for calf birth weight, weaning weight and metabolic profile. A lower *mRNA* expression of *MYH1* was observed ($P = 0.02$) in skeletal muscle of calves from the HME and LME groups compared to the CME. An increased mRNA expression of both *MYH2a* ($P = 0.04$) and *MYH2x* ($P = 0.01$) was observed in calves from LME compared to HME, suggesting potential alterations in muscle fiber composition that may influence metabolic efficiency and growth performance. A greater *mRNA* expression of *PPARα* ($P = 0.04$), *PPARGC1α* ($P = 0.04$) and *MEF2A* ($P = 0.01$) were observed in calves from the LME group compared to the HME. A greater AMPK activity was observed ($P = 0.01$) in skeletal muscle of calves from LME group compared to CME and HME.

In contrast, Akt activity was greater in HME and LME groups compared to CME (P = 0.01). Our findings suggest that maternal ME intake affected the muscle energy metabolism of the offspring, the oxidation of fatty acids, mitochondrial biogenesis and the use of muscle fiber type fuel.

Key words: energy metabolism, fetal programming, maternal nutrition, metabolic flexibility, metabolizable energy, skeletal muscle

INTRODUCTION

Proper maternal nutrition during late gestation is crucial for fetal development and long-term productivity of the offspring (Funston et al., 2010; Costa et al., 2021; Santos et al., 2022). While previous research indicates that increasing maternal energy density during late gestation may be useful to improve the energy status of cows (Barcelos et al., 2022; Waldon et al., 2023), a low-energy diet negatively impacts the growth and development of neonatal calves (Chen et al., 2022). Other studies have shown that energy restriction during early development can alter muscle and blood transcriptomes, potentially affecting muscle development and metabolic pathways (Sanglard et al., 2018; Costa et al., 2021; Nascimento et al., 2024).

Maternal nutrition during gestation can also influence the skeletal muscle metabolism of offspring, driving structural and functional changes in muscle that support increased metabolic flexibility postnatally (Norman et al., 2012; Carvalho et al., 2022). While muscle fiber types begin to form in the embryonic phase, their phenotype can change after birth. This ability of skeletal muscle to adjust its energy metabolism is referred to as "fiber plasticity" (Talbot and Maves, 2016), which allows the tissue to switch between fuel sources. The regulation of this process involves signaling pathways that control key aspects of metabolism, including glucose utilization, lipid oxidation, and mitochondrial biogenesis (Lin et al., 2002). This inability to rapidly adjust energy substrate utilization has been termed metabolic inflexibility and has recently been associated with metabolic chronic diseases (Shoemaker et al., 2023). Studies in

rodents have shown that nutrient restriction during gestation can enhance oxidative metabolism in offspring under ad libitum conditions. However, it may also impair their ability to adapt fuel usage in skeletal muscle (Aragão et al., 2014). Additionally, offspring born to nutrient-restricted dams showed gene expression patterns in skeletal muscle that promote the transport of long-chain fatty acids into mitochondria, indicating a preference for specific carbon sources for energy production (Aragão et al., 2014). These findings indicate that maternal nutrition exerts a programming effect on offspring skeletal muscle metabolism, potentially influencing the preferential utilization of specific carbon substrates for energy production. Consistent with this, Meneses et al. (2024) demonstrated that protein supplementation during mid-gestation enhances fetal growth and optimizes nutrient utilization, thereby further substantiating the role of maternal dietary composition in the metabolic programming of offspring. However, investigations into these mechanisms in cattle remain limited and additional research is warranted to elucidate the effects of maternal energy intake on the developmental programming of skeletal muscle energy metabolism in offspring.

In this study, we hypothesize that maternal metabolizable energy intake during late gestation programs skeletal muscle metabolism in offspring by modulating the expression of genes and key proteins involved in oxidative metabolism and muscle fiber type. Specifically, we propose that energy intake restriction enhances fatty acid oxidation and the expression of oxidative markers in offspring skeletal muscle, in comparison to offspring born to dams fed at maintenance and above maintenance levels during late gestation. As such, this study aims to explore how varying levels of maternal metabolizable energy intake during late gestation impact the skeletal muscle energy metabolism in beef offspring.

MATERIAL AND METHODS

All procedures used in this study were approved by the animal care committee at University of Guelph (Animal Utilization Protocol No. 4419) and followed the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Animals, treatments and experimental design

Forty-two pregnant Angus-Simmental beef cows (680.8 ± 74.4 kg), including primiparous (n = 21) and multiparous (n = 21), were utilized and housed at the Ontario Beef Research Center (OBRC) at the University of Guelph. Cows were artificially inseminated with semen from seven Black Angus bulls of similar genetic merit. Heifers were bred to begin calving two weeks earlier than the cows. Multiparous cows had between two and three previous parturitions. Cows were blocked by predicted calving date based on an estimated gestation period of 282 days and treatments were balanced by initial body weight, sire and parity.

Experimental diets (Table 1) were formulated using CNCPS 6.55 (Nutritional Dynamic System software, RUM&N Sas, Via Sant' Ambrogio, Italy) to deliver the specified metabolizable energy (ME) levels while meeting or exceeding metabolizable protein (MP) requirements (Van Amburgh et al., 2015a,b; Higgs et al., 2015). Model inputs were based on herd averages from the Ontario Beef Research Center, encompassing both heifers and cows. This approach reflects common industry practices in Canadian cow–calf systems and addressed logistical constraints that prevented rationing by parity. Predicted ME and MP requirements were calculated using a reference model cow: a 715 kg Angus female with a body condition score (BCS) of 7, programmed to lose 2 BCS units over the final 100 days of gestation and calving a 38 kg calf. This scenario represents a planned moderate negative energy balance and served as the baseline to define 100% of ME requirements. Accordingly, cows were randomly assigned to one of three dietary treatments designed to provide 92% (LME, n = 16), 104%

(CME, n = 13), or 118% (HME, n = 13) of the predicted ME requirements. Therefore, CME and HME represent relative increases over a restricted baseline, not necessarily indicative of BCS maintenance or gain.

Experimental diets began 53 ± 0.4 days before expected calving. Cows were housed by treatment and parity in pens of six animals, sharing three Insentec feeders (Roughage Intake Control System, Insentec, Marknesse, the Netherlands), and were limit-fed at 1.35% (LME), 1.4% (CME), or 1.45% (HME) of body weight on a dry matter basis. Feed quantities were adjusted weekly based on ingredient dry matter and cow body weight. Prior to diet implementation, cows underwent a 2-week adaptation period receiving the CME diet to acclimate to limit feeding. Postpartum, all cows were fed a common lactation diet ad libitum.

Calving occurred during spring (early February to early May). After birth, cow–calf pairs were moved to individual maternity pens for 2 days to support bonding, then housed in groups with access to a creep pen for clean bedding. Calves suckled ad libitum until weaning. At 60 days postpartum, pairs were moved to pasture as a single herd, grazing on a mix of grasses and legumes. Calves were weaned at 209 days of age.

Forages were sampled twice weekly, concentrates were sampled every two weeks, and total mixed rations were sampled weekly. Feed samples were dried in a gravity convection oven at 60°C for two consecutive days (dry forages and concentrates) or until consistent weights were recorded across two calendar dates (ensiled forages). Dry matter analysis of feed ingredients was used to adjust rations for any changes in dry matter using a running average across the study. After the study, dried samples were ground through a 1-mm screen and were sent to A&L Canada Laboratories Inc. (A&L Canada Laboratories Inc., London, ON) for wet chemistry analysis. Samples were analyzed using the AOAC methodology (Official Methods of Analysis, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured with the Ankom 200 using the Ankom Method 6 and Ankom Method 5 procedures (AOAC 2002.04,

AOAC 973.18). Crude protein (CP) was measured by combustion with the LECO FP628 nitrogen analyzer (AOAC 990.03). Crude fat (EE) was measured with petroleum ether as a high temperature solvent to extract fat and oil (AOAC 920.39). Starch was measured using heat stable amylase and amyloglucosidase with the Megazyme K-TSTA method (AOAC 996.11). Sugar was measured using sulphuric acid/phenol and 80% ethanol:water using the colorimetric method.

Animal data and sample collections

Cows' initial body weight (IBW) was collected on two consecutive days at treatment enrollment, on days -54 ± 0.4 and -53 ± 0.4 relative to parturition. Final body weight (FBW) was measured on day -3 ± 0.4 before parturition. Initial subcutaneous fat thickness (ISFT) was measured at both the rib and rump using ultrasonography with an EXAGO ultrasound machine and a linear transducer (#L3180B, 18 cm, 3.5 MHz frequency, Echo Control Medical, Angoulême, France) on day -54 ± 0.4 relative to parturition. Additionally, final subcutaneous fat thickness (FSFT) was measured again at the rib and rump on day 13 ± 0.3 after parturition. The Rib fat thickness was measured at the ¾ end of the ribeye area, using a image collected between the 12th and 13th ribs. The rump fat thickness was assessed at the rump, between the ischium and the pubis. Ultrasound image analysis was performed using ImageJ (National Institutes of Health, Maryland, USA). Initial and final body weights (BW) were used to calculate total dry matter intake (DMI) and as a percentage of body weight during the prepartum period. Similarly, body weight recorded after parturition and on day 55 ± 0.3 postpartum was used to calculate total DMI and intake as a percentage of body weight during the postpartum period. The variation in subcutaneous fat thickness was obtained relative to the measurements at the prepartum period as follows:

$$VSFT = \frac{FSFT - ISFT}{ISFT}\ x\ 100$$

Where VSFT (%) = variation in subcutaneous fat thickness; FSFT = final subcutaneous fat thickness; and ISFT = initial subcutaneous fat thickness.

At birth, calves were weighed before suckling the dam, and again on day 28 ± 0.18, and day 209 ± 1.38 at weaning. The birth and weaning weights were used to calculate the average daily gain (ADG) of the calves during the suckling phase, body weight gain from birth to weaning divided by days of age at weaning. At 28 days of age, blood samples were collected from the calves by jugular venipuncture in 10 mL green top heparinized saline vacutainer tubes and red top vacutainer tubes. (BD Vacutainer®, BD and Company, Franklin Lakes, New Jersey, USA). Aprotinin (Aprotinin from bovine lung, Sigma Aldrich, St. Louis, Missouri, USA) was added to plasma samples at a ratio of 0.5 µL per 1 mL of blood. These samples were immediately placed on ice after collection. Serum samples were allowed to clot at room temperature. Plasma and serum samples were centrifuged at 2500 × g (4º C) for 15 minutes. After centrifugation, supernatant from the samples was separated and stored at -20º C until analysis.

At 30 days of age Longissimus dorsis muscle samples (located between the 12th and 13th ribs) were biopsied from the calves. The biopsy site was initially shaved and sanitized with 4% chlorohexidine and 70% ethanol alcohol. Following the sanitization, the biopsy site was anesthetized with 2% lidocaine (7 mL). A 1.5-cm incision was made through the skin using a 14-blade scalpel and muscle tissue was excised by using an 8mm biopsy punch and rinsed with 0.9% Saline solution. The skin was sealed using veterinary surgical glue (VetBond, 3M). The muscle samples were rinsed with a sterile saline solution, placed in a 2mL cryotube, and immediately snap-frozen in liquid nitrogen for transport to the lab. In the lab, the samples were powdered in liquid nitrogen using a pre-cooled mortar and pestle, transferred to a pre-cooled cryotube, and stored at -80ºC for future nucleic acid and protein extraction.

Plasma and serum analyses

Serum samples were analyzed for the Bovine Metabolic Profile at University of Guelph's Animal Health Laboratory (Animal Health Laboratory, University of Guelph, Guelph, ON) photometric analysis using an autochemistry analyzer (Cobas 6000 c501, Roche Diagnostics, Indianapolis, IN) including glucose, beta-hydroxybutyrate (BHBA) and non-esterified-fatty-acids (NEFA).

Plasma samples were analyzed for insulin using the ALPCO Bovine Insulin ELISA kit (ALPCO, Salem, NH, USA) following the manufacturer's instructions: plates were read once at 450 nm using an absorbance reader (Cytation 5, BioTek, Winooski, VT, United States). Samples were analyzed in duplicate with and inter-assay CV of 4.6% and an intra-assay CV of 2.9%.

Total RNA extractions and mRNA expression analyses

Total RNA was extracted from 0.1 g of tissue using Trizol® (InvitrogenTM, Thermo Fisher Scientific®, Oregon, USA) following the manufacture's recommendations. The total RNA was quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific®, Waltham, MA, USA), ensuring an optimal 260/280 ratio between 1.8 and 2.0, and the integrity was assessed in a 1% agarose gel. The RNA samples were reverse transcribed into cDNA using the GoScriptTM Reverse Transcription System Kit (Promega, Madison, WI, USA). The primers (Table 2) for amplification of target and endogenous genes were designed using PrimerQuest Software (PrimerQuest–design qPCR assays | IDT [idtdna.com]) with sequences obtained from GenBank (GenBank Overview [nih.gov]). Real-time quantitative PCR was performed in the thermal cycler QuantStudio 3 (Applied Biosystems, Foster City, CA, USA) using the SYBR Green detection method (Applied Biosystems, Foster City, CA, USA) and SYBR™ Green PCR

Master Mix (InvitrogenTM, Thermo Fisher Scientific®, Oregon, USA). The amplification efficiency was 0.90–0.99. After amplification, a melting curve (0.01 °C/s) was used to confirm product purity and to confirm the specificity of the primers, ensuring that only the intended target sequence was amplified. Results of gene expression were calculated according to the methods described by Steibel (2009).

Protein extraction and Western Blotting analyses

Total protein was extracted from 0.1 g of tissue in 1 mL of lysis buffer (10 mM of Tris HCl [pH 7.6], 150 mM of NaCl, 1% of Triton X-100, 0.5% sodium deoxycholate, 1% sodium dodecyl sulfate [SDS], and 1% of protease inhibitor cocktail mammalian cells and tissues [Sigma-Aldrich®]), and the lysate was sonicated. Total protein content was estimated using the Bradford protein assay (Bio-Rad, Hercules, CA, USA), aliquoted and stored at −80°C. The proteins were separated using a 10% SDS-PAGE gel loaded with 40 μg of protein per sample, transferred to a 0.45 μm Nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) using a semi-dry Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, CA, USA), and blocked for 1 h at room temperature with 3% bovine serum albumin (BSA, Sigma Aldrich®) in 1x Tris-Buffered Saline (TBS1x; 50mM Tris-HCL, pH 7.5; 150mM NaCl; Sigma Aldrich®). Subsequently, the membranes were incubated for 12 h at 4°C with the primary antibodies (Table 3) diluted in blocking solution. After 12 h of incubation, membranes were washed 3 times 5 minutes with Tris Buffered Saline and 0.1% Tween® (TBSt) and incubated with the secondary antibody (Table 3) diluted in blocking solution for 1 h at room temperature. Membranes were then washed with TBSt 3 times 7 minutes, revealed by ECL Plus Western Blotting Detection System (GE HealthCare, Buckinghamshire, Ul) and the images generated by ChemiDoc XRS+ System (Bio-Rad, Hercules, CA, USA) and evaluated using Image Lab Software (Bio-Rad, Hercules,

CA, USA). The α-tubulin was used as a housekeeping protein to normalize the abundance of the target proteins.

Statistical analysis

The dam and calf performance traits, and calf metabolism traits were analyzed according to the following mixed model:

$$Y_{jklm} = \mu + T_j + P_k + S_l + e_{jklm}$$

[Eq. 1]

where $Yjklm$ is the observed value; μ is the intercept; Tj is the fixed effect of the *j*th level of maternal dietary treatment, with *j* = 1…3 Pk is the fixed effect of the *k*th level of parity, with *k* = 1…2; Sl is the random effect of the *l*th Sire, with *l* = 1…7 assuming $S \sim N(0, \boldsymbol{IS}\sigma S2)$, where $\boldsymbol{IS}$ represents the identity matrix of dimension equal to the number of sires; and $ejklm$ is the random error associated with $yjklm$, assuming $e \sim N(0, \boldsymbol{Ie}\sigma e2)$ where $\boldsymbol{Ie}$ represents the identity matrix of dimension equal to the number of observations. Prior to final analyses, residuals were evaluated for normality. For each analysis, data points were removed one at a time until absolute Studentized residuals were lower than 3 and had non-significant ($P > 0.01$) Shapiro-Wilk's test for normality. Expected means were generated from the final models and separated using Tukey's test when different at $P \leq 0.05$.

The protein abundance data were analyzed using a Poisson mixed model using a log-link function:

$$Y_{jklm} = e^{\mu + T_j + P_k + S_l}$$

[Eq. 2]

where e is the exponential, and $Yjklm$, μ, Tj, Pk, and Sl are as previously defined in Eq. [1]. Expected rates were generated from the final models and separated using Tukey's test when different at $P \leq 0.05$.

Prior to the analysis of the RT-PCR data, the CT values were adjusted ($adjCT$) for their respective primer efficiency and back-transformed to the log2 scale as:

$$adjCT = \log_2(E^{-CT})$$

[Eq. 3]

where E=1+Eff, where Eff represents the calculated primer efficiency. Afterwards, the $adjCT$ data were analyzed according to the linear mixed model below, following the strategy proposed by Steibel et al., 2009 where the data on the target and endogenous control genes are analyzed simultaneously:

$$adjCT_{ijklm} = \mu + G_i + T_j + (G * T)_{ij} + P_k + (G * P)_{ij} + S_{il} + A_{jklm} + e_{ijklm}$$

[Eq. 4]

where μ, Tj, and Pk are as previously defined, whereas $adjCTijklm$ is the adjusted CT as in Eq. [3], Gi is the fixed effect of the *i*th level of gene, with *i* = 1,2; $(G*T)ij$ is the fixed effect for the interaction between Gi and Tj; $(G*P)ik$ is the fixed effect for the interaction between Gi and Pk; Sil is the random effect of the *l*th Sire on each gene, assuming S~$N(0,\boldsymbol{IGS}\sigma Si2)$, where $\boldsymbol{IGS}$ represents the identity matrix with dimensions equal to the number of sires times the number of genes (*i.e.*, 2); $Ajklm$ is the random effect of the *m*th animal, assuming A~$N(0,\boldsymbol{IA}\sigma Si2)$, where $\boldsymbol{IA}$ represents the identity matrix with dimensions equal to the number of animals; and $eijklm$ is the random error associated with $adjCTijklm$, assuming e~$N(0,\boldsymbol{IGe}\sigma e2)$, where $\boldsymbol{IGe}$ represents the identity matrix with dimensions equal to the number of observations across the two genes. The interactions between Gi with Tj and Pk, respectively, were used to assess the effects of *Treatment* and *Parity*, respectively, through orthogonal contrasts, as in Steibel et al. (2009). Expected $adjCT$ were computed as $-\Delta adjCT$, such that the differences between the levels of *Treatment* and *Parity* were estimated as $-\Delta\Delta adjCT$, and then used to compute the *Gene Ratio Expression* (GRE) as:

$$GRE = 2^{-\Delta\Delta CT}$$

[Eq. 4]

Estimates of *GREs* were separated using contrasts when different at $P \leq 0.05$. All analyses were performed in SAS Studio 3.81 (Enterprise Edition, SAS Institute Inc., Cary, NC, USA).

RESULTS

Cow-calf performance and calf metabolic parameters

The initial body weight (IBW; $P = 0.26$) did not differ among treatments. However, final body weight (FBW; $P = 0.01$) was greater in the HME and CME compared to the LME cows (Table 4). The initial subcutaneous fat thickness at the 12th and 13th ribs (ISFT-rib; $P = 0.40$) and at the rump (ISFT-rump; $P = 0.12$) did not differ between treatments (Table 4). However, final subcutaneous fat thickness at the rib (FSFT-rib; $P = 0.01$) and at the rump (FSFT-rump; $P = 0.04$) was greater in the HME compared to LME cows. The variation in subcutaneous fat thickness at the rib (VSFT-rib; $P = 0.01$) and at the rump (VSFT-rump; $P = 0.05$) was lower in HME than in LME cows (Table 4). The average daily gain (ADG; $P = 0.01$) before calving was lower in LME compared to CME and HME cows (Table 4). During the prepartum period, cows fed the HME and CME diets had greater ($P < 0.01$) dry matter intake (DMI) than those fed the LME diet, as designed. Prepartum DMI per percentage of body weight percentage (%BW, $P < 0.01$) was greater in cows fed the HME and CME diets than in those fed the LME diet (Table 4). In the postpartum period, total DMI ($P = 0.01$) and DMI per %BW ($P = 0.02$) were higher in the CME treatment compared to HME (Table 4).

No differences were observed among treatments for calf birth weight ($P = 0.52$), weaning weight ($P = 0.13$), or ADG ($P = 0.75$) as shown in Table 4. No differences were observed between experimental treatments on serum levels of insulin ($P = 0.47$), glucose ($P = 0.92$), β-hydroxybutyrate (BHBA; $P = 0.72$), and non-esterified fatty acids (NEFA; $P = 0.68$) in calves at 28 days of age (Table 5).

mRNA expression of energy metabolism in skeletal muscle of the offspring

The mRNA expression of *IRS1* ($P = 0.74$) and *SLC2A4* ($P = 0.92$) in skeletal muscle of the offspring did not differ between treatments, while a greater mRNA expression of *MEF2A* was observed in LME group compared to HME and CME groups ($P < 0.01$; Table 6). The mRNA expression of *MYH1* was decreased ($P = 0.02$) in skeletal muscle of calves from the HME and LME groups compared to the CME group. In contrast, the mRNA expression of *MYH7* tended to be greater ($P = 0.07$) in skeletal muscle of calves from LME compared to CME group. An increased mRNA expression of both *MYH2a* ($P = 0.04$) and *MYH2x* ($P = 0.01$) was observed in skeletal muscle of calves from LME compared to HME group (Table 6).

A greater mRNA expression of *PPARα* ($P = 0.04$) and *PPARGC1α* ($P = 0.04$) was observed in skeletal muscle of calves from the LME group compared to the HME group, without differences for *PPARγ* ($P = 0.98$) (Table 6). For mRNA expression of markers of oxidative and lipid metabolism in skeletal muscle tissue of offspring, including *SREBF1 (*$P = 0.19$), *ACACA (*$P = 0.28$*), FASN (*$P = 0.80$*), CPT1α (*$P = 0.80$*), and UCP3 (*$P = 0.45$*),* no differences were found among treatments (Table 6).

AMPK and Akt activity

The p-AMPK/AMPK abundance ratio was greater ($P < 0.01$) in skeletal muscle of calves born to dams fed LME diet compared to those born to dams fed CME and HME diets (Figure 1). In contrast, the abundance ratio of p-Akt/Akt was greater in skeletal muscle of calves born to dams fed both the HME and LME diets compared to those born to dams fed CME diet ($P < 0.01$; Figure 2).

DISCUSSION

The objective of the present study was to investigate how metabolizable energy (ME) intake during late gestation influences the skeletal muscle energy metabolism of offspring in beef cattle. Our results demonstrated that variations in cow performance were impacted by the level of ME intake, validating the effectiveness of the experimental treatments. As intended, all cows were managed under a negative energy balance during late gestation. Although dietary treatments were formulated to provide 92%, 104%, and 118% of predicted ME requirements, it is important to note that the reference model used to estimate ME requirements assumed a 715 kg Angus cow with BCS 7, programmed to lose 2 BCS units over the last 100 days of gestation. Thus, even cows fed 118% of predicted requirements were not necessarily in positive energy balance. In fact, all cows, including those in the HME group, mobilized subcutaneous fat as objectively measured by ultrasound, confirming a negative or near-balanced energy state. Although HME and CME cows showed positive average daily gains, these gains are largely attributable to gestational tissue accretion (e.g., fetus, uterus, fluids), which is estimated at approximately 0.5 kg/day in late gestation (Gionbelli et al., 2015). These differences in maternal performance were crucial for enabling the investigation of calf energy metabolism, which was the primary goal of this study. As such, the subsequent discussion will focus exclusively on the evidence supporting the notion that maternal ME intake may impact the energy metabolism of the offspring.

Previous studies have suggested that the effects of maternal nutrition can have long-lasting effects, during the calf early development until adulthood even when changes in birth weight are not observed (Lukaszewski et al., 2013; Du, 2023; Nascimento et al., 2024). In the current study, no differences in birth weight were observed among the treatment groups, indicating that overall fetal growth was not affected. However, the activity of AMPK (AMP-activated protein kinase), a key enzyme involved in regulating cellular energy metabolism, was

influenced by maternal diet. AMPK is primarily activated under conditions of low ATP levels and an increase in AMP/ADP, playing a critical role in modulating muscle metabolism by promoting fatty acid oxidation and glucose uptake (Cantó and Auwerx, 2009). In the current study, calves from the LME group showed higher AMPK activation compared to those from the HME and CME groups. This difference occurred despite all calves being managed as a single herd, under *ad libitum* feeding conditions, and with no significant variations in their blood metabolic profiles. This observation suggests that maternal ME intake, particularly at 92% of the required ME during late gestation, may affect the metabolic flexibility of skeletal muscle in the offspring.

AMPK regulates the expression of the transcription factors *PPARGC1α* and *PPARα*, which form a complex that promotes the expression of various genes involved in fatty acid oxidation and mitochondrial function (Liang and Ward, 2006). The greater mRNA expression of *PPARα* and *PPARGC1α* in the skeletal muscle of calves born to cows from the LME group compared to the HME group, along with greater AMPK activation, suggests the increase in beta-oxidation, despite the lack of differences in blood NEFA concentrations between these groups. Moreover, the *CPT1α* which is involved in promoting fatty acid oxidation for energy production, and *UCP3* which is involved in mitochondrial efficiency (Hoeks et al., 2006; Bougarne et al., 2018) are also both regulated by *PPARGC1α* and *PPARα* (Song et al., 2004; Villarroya et al., 2007). In our study, we did not identify differences for *CPT1α or UCP3* between the treatments. Therefore, our finds suggest that the higher expression of *PPARGC1α* and *PPARα* did not necessarily reflect an increase in the flux of long-chain fatty acids into mitochondria and protective effects due this mechanism. Our findings partially corroborate those found by Aragão et al. (2014), where the offspring born to dams with protein restriction during pregnancy showed greater activation of AMPK, and greater expression of *PPARGC1α*, *PPARα*, *CPT1α* and *UCP3* in *ad libitum* conditions. In addition, the activation of AMPK and

greater expression of *PPARGC1α* may lead to more efficient individuals in terms of animal productivity, since studies have indicated that high mitochondrial respiratory activity and AMPK phosphorylation are present in individuals with high feed efficiency (Bottje and Kong, 2013). Previous studies have shown that mitochondria from the muscle of more efficient animals (low residual feed intake) exhibit reduced production of reactive oxygen species compared to less efficient animals (with high residual feed intake) (Grubbs et al., 2013). Additionally, the mitochondrial protein profile in more efficient animals revealed enhanced antioxidant defenses (Grubbs et al., 2013). This indicates a lower susceptibility to oxidative stress, reducing the need for cellular repair and enabling a greater allocation of dietary energy toward growth (Rauw et al., 2025). Furthermore, feed efficiency is closely linked to metabolic flexibility, the ability of an organism to adapt fuel oxidation to fuel availability, which allows for optimized nutrient utilization and energy allocation toward growth (Rauw et al., 2025). In this sense, our findings suggest that calves born to dams fed 92% of ME at late gestation have change the energy metabolism of their skeletal muscle by increasing the fatty acid oxidation pathway, which could lead to changes in animal performance later in life.

PPARGC1α, a key regulator of energy metabolism and mitochondrial biosynthesis in skeletal muscle, influences muscle development and fatty acid oxidation by modulating mitochondrial number and respiration (Hood et al., 2006). However, impaired mitochondrial function, reduced biogenesis, and increased oxidative damage can negatively affect muscle development (Zou et al., 2016). Notably, calves born to cows that consumed 118% of their metabolizable energy (ME) requirements during late gestation exhibited reduced *PPARGC1α* expression. This observation aligns with findings from Zou et al. (2017), which reported that the expression of several mitochondrial function-related genes was significantly lower in fetuses from overfed dams, suggesting compromised skeletal muscle mitochondrial function. Furthermore, mitochondrial dysfunction resulting from maternal overnutrition has been

associated with increased oxidative stress in offspring. This increased oxidative stress can further impair growth by diverting energy away from muscle development and toward cellular repair mechanisms, thereby reducing the availability of dietary energy for productive purposes (Zou et al., 2017). In addition, the reduced expression of *PPARGC1α* leads to lower muscle mass and a higher proportion of small myofibers (Ma et al., 2022). Given its essential role in muscle metabolism, variations in *PPARGC1α* expression can therefore directly impact growth efficiency.

The *PPARGC1α* is linked to muscle tissue plasticity, with higher mRNA expression associated with the transition from glycolytic to oxidative muscle fibers (Lin et al., 2002). In the LME group, which showed elevated mRNA expression of *PPARGC1α* in skeletal muscle, a higher proportion of oxidative muscle fibers was expected compared to the HME group. However, no differences in mRNA expression of *MYH7* were observed between the groups, though both LME and HME groups showed greater mRNA expression of *MYH7* compared to the CME group. Additionally, the lower mRNA expression of *MYH1* in both the LME and HME groups compared to the CME group suggests that meeting 92% and 118% of maternal predicted ME requirements can trigger mechanisms that alter skeletal muscle fuel usage. A greater mRNA expression of *MYH2a* and *MYH2x* was found in the LME group compared to the HME group. These genes are responsible for type IIa muscle fibers, which can switch between glycolytic and oxidative fuel use (Norman et al., 2012). Maternal undernutrition has been shown to decrease type II muscle fibers and increase type muscle I fibers in ewes (Fahey et al., 2005). Our findings suggest a similar mechanism in calves born to dams from the high ME group, with a reduction in type IIa muscle fibers and impaired oxidative metabolism, indicating reduced metabolic flexibility in the skeletal muscle of calves from this group.

Another key regulator of muscle energy metabolism is the protein kinase B (Akt) pathway, which plays a critical role in promoting protein synthesis (Schiaffino et al., 2013).

One of the ways Akt is activated is through insulin and its receptor, which can stimulate glucose uptake via SLC2A4 (Long et al., 2012). In our study, the insulin and glucose blood levels, and the mRNA expression of insulin receptor subunit 1 (*IRS1*) and *SLC2A4* did not differ among the groups, suggesting another pathway might be responsible for Akt activation in HME group. Although AMPK and Akt were initially thought to have an antagonistic relationship, recent studies suggest that chronic AMPK activation can trigger Akt activation as a compensatory mechanism to regulate protein synthesis (Shoemaker et al., 2023). This may explain the increased Akt activation observed in the LME group in our study. In fact, in association with increased Akt activation, we also observed an increased mRNA expression for *MEF2A* in LME group compared to CME and HME. The *MEF2A* (myocyte enhancer factor isoform 2A) is an important transcription factor involved in muscle mass control and hypertrophy (Schiaffino et al., 2021), and can be regulated by Akt and AMPK to further control muscle development (Chen et al., 2020). Overall, the increased Akt activation and mRNA expression of MEF2A in the skeletal muscle of calves born to dams in the LME group may indicate a compensatory mechanism in response to elevated AMPK activation, helping to mitigate the effects of reduced anabolic processes caused by AMPK activation.

CONCLUSION

Our findings suggest that calves born to dams fed 92% of the predicted ME requirement show a metabolic adaptation that favors fatty acid oxidation for muscle energy production. The greater mRNA expression of markers for type I and IIa muscle fibers in agreement to increased expression of oxidative metabolism genes may provide an advantage in terms of energy flexibility and in future productive efficiency. Conversely, calves born to dams fed 118% of the predicted ME requirement decreased expression of oxidative metabolism markers, suggesting potential impairments in mitochondrial function and metabolic flexibility in skeletal muscle.

Lastly, our findings suggest that excessive maternal energy intake can cause mitochondrial dysfunction and elevate oxidative stress in the offspring's skeletal muscle, which may ultimately hinder muscle development and growth efficiency later in life.

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TABLES

Table 1. Ingredient composition, chemical characteristics, and feeding rates of prepartum diets providing 118% (HME), 104% (CME), and 92% (LME) of predicted metabolizable energy (ME) requirements for pregnant cows from day -53 to calving, and ad libitum postpartum lactation diet.

Item	Experimental treatments			
	HME	CME	LME	LACTATION
Diet composition, %DM				
Haylage	5	5	5	32.1
Corn Silage	5	5	5	25.2
Chopped Dry Hay	44.5	37.5	30.5	0
Wheat Straw	5	30	55	37.8
High Moisture Corn	18.5	18.5	0	0
Whole Corn	18.5	0	0	0
Urea	0	0.5	1	0
Vitamin and Mineral Premix[1]	3.5	3.5	3.5	5.0
DM, %	80.76	80.86	84.53	51.1
Chemical composition, %DM				
Crude protein	10.83	10.62	10.58	9.6
aNDFom	36.78	51.91	67.38	54.93
Starch	26.98	14.95	2.87	9.29
Ether extract	3.02	2.57	2.16	2.68
Ca	0.66	0.66	0.66	0.68
P	0.41	0.34	0.27	0.32
Mg	0.26	0.25	0.24	0.27
K	1.64	1.57	1.51	1.44
Predicted ME Supply, Mcal/kg	2.47	2.26	2.14	1.92
Predicted ME Supply, % requirement	117.5	104.1	92.4	151.3
Predicted MP Supply, % requirement	124.9	120.5	113.7	166

[1] The mineral-vitamin premix was formulated by Floradal Feed Mill Limited (Floradale, ON); chemical composition: 293.4 kIUU/kg vit A, 67.1 kIU/kg vit D, 1364.6 kIU/kg vit E, 447.0 ppm Cu, 1586.4 ppm Fe, and 3866.6 ppm Zn

Table 2. List primers for mRNA expression by RT-qPCR.

Gene symbol	NCBI accession number	Primer
Target genes		
ACACA	NM_174224.2	F: CTCCAACTTCCTTCACTCCTTAG R: ACATACTTCACTCCCTCGTAGA
CPT1A	NM_001304989.2	F: CCCAAATCATGCACTGTTGAC R: CCTGAGAGACAAGCCCAAATAG
FASN	NM_001012669.1	F: AGCACACGCCTGTAGTATTC R: TTCCAGAAGCCGCACTTT
IRS1	XM_003585773.6	F: GCCTATGCCAGCATCACTTT R: GGAGGATTTGCTGAGGTCATTT
MEF2A	NM_001083638.2	F: TCCACCTCAAGCCACATTAC R: GAGGTCTGAAGTGCTCAACAT
MYH1	NM_174117.1	F: GTACGTGAACTGGAAGGAGAAG R: AAATCCTGGAGCCTGAGAATG
MYH2a	NM_001166227.1	F: AGAGCAGAGGATGAGGAAGA R: GTCAGCTCAAGGTCGTCTATG
MYH2x	XM_010816053.4	F: CCTTACCTCCGAAAGTCTGAAA R: CTGCTCTGGATAGTCCCTTTG
MYH7	NM_174727.1	F: CAAGGGCTTGAATGAGGAGTAG R: GCTTTATTCTGCTTCTTCCAAAGG
PPARα	NM_001034036.1	F: CCTACGGGAATGGCTTCATAAC R: GCAGCCACAAAGAGGGAAATA
PPARγ	NM_181024.2	F: TTATTCCCACCTCCTCCAAAC R: CACGACTCCCACCGATATTT
PPARGC1α	NM_177945.3	F: ACACCAAACCCACAGAGAAC R: GGGATGACCGAAGTGCTTATT
SLC2A4	NM_174604.1	F: CTTGGTCCTTGGCGTATTCT R: CAGGTCTCATTGTAGCTCTGTT
SREBF1	NM_001113302.1	F: GACTACATCCGCTTCCTTCAG R: CCAGGTCCTTCAGCGATTT
UCP3	NM_174210.1	F: GGTGAAGACGCGGTATATGAA R: CAAAGCACTGAAGACCACAATC
Endogenous gene		
18s	NM_001304989.2	F: CCCAAATCATGCACTGTTGAC R: CCTGAGAGACAAGCCCAAATAG

Table 3. List of antibodies used in Western blotting analysis

Antibody	Host	Dilution	Manufacturer	Catalog number
Primaries antibodies				
Akt	Rabbit Monoclonal IgG	1:1000	Cell signaling	9272S
p-Akt	Rabbit Monoclonal IgG	1:1000	Cell signaling	9271S
AMPK	Rabbit Monoclonal IgG	1:1000	Cell signaling	2532S
p-AMPK	Rabbit Monoclonal IgG	1:1000	Cell signaling	2535S
α-tubulin	Rabbit Monoclonal IgG	1:1000	Cell signaling	2144S
Secondary antibodies				
HRP	Goat anti-Rabbit IgG	1:5000	Thermo Fisher Scientific®	A16096

Table 4. Least-square means ± SEM for the performance of cows and calves born to dams fed prepartum diets providing 118% (HME), 104% (CME), and 92% (LME) of the predicted metabolizable energy (ME) requirements for pregnant cows from day -53 to calving, followed by an ad libitum postpartum lactation diet.

Item	**Experimental treatments**			***P*-value**
	HME	**CME**	**LME**	**Treatment**
Cow performance				
Initial body weight, kg	664.5 ± 18.9	706.9 ± 18.9	673.5 ± 16.9	0.26
[1]Final body weight, kg	713.8 ± 6.4 [a]	710.8 ± 6.0 [a]	672.4 ± 5.8 [b]	0.01
Initial subcutaneous rib fat thickness, mm	9.29 ± 0.85	8.26 ± 0.84	7.75 ± 0.75	0.40
Final subcutaneous rib fat thickness, mm	7.30 ± 0.41 [a]	7.31 ± 0.41 [a]	5.86 ± 0.37 [b]	0.01
Variation in subcutaneous rib fat thickness, %	-14 ± 0.05 [a]	-6 ± 0.05 [a]	-32 ± 0.04 [b]	0.01
Initial subcutaneous rump fat thickness, mm	16.42 ± 1.51	13.29 ± 1.49	12.31 ± 1.32	0.12
Final subcutaneous rump fat thickness, mm	13.51 ± 0.48 [a]	12.51 ± 0.49 [ab]	11.50 ± 0.45 [b]	0.04
Variation in subcutaneous rump fat thickness, %	-5 ± 0.01 [a]	-11 ± 0.05 [ab]	-19 ± 0.05 [b]	0.05
Average daily gain prepartum, kg/day	0.63 ± 0.12 [a]	0.57 ± 0.12 [a]	-0.19 ± 0.11 [b]	0.01
Dry matter intake prepartum, kg/day	9.81 ± 0.15 [a]	9.75 ± 0.15 [a]	8.48 ± 0.11 [b]	0.01
Dry matter intake prepartum, %BW	1.48 ± 0.02 [a]	1.45 ± 0.02 [a]	1.29 ± 0.02 [b]	0.01

Dry matter intake postpartum, kg/day	14.95 ± 0.48 [b]	17.13 ± 0.50 [a]	15.64 ± 0.46 [ab]	0.01
Dry matter intake postpartum, %BW	2.15 ± 0.08 [b]	2.40 ± 0.08 [a]	2.29 ± 0.08 [ab]	0.02
Calf performance				
Birth weight, kg	34.67 ± 1.3	35.28 ± 1.3	33.42 ± 1.2	0.52
Weaning weight, kg	302.62 ± 9.57	275.11 ± 9.18	283.86 ± 8.04	0.13
ADG to 209 days, kg/day	1.23 ± 0.06	1.18 ± 0.06	1.21 ± 0.06	0.75

[1]*Adjusted for initial body weight*

[2] Final body weight (FBW) and subcutaneous fat thickness (FSFT) were recorded three days prior to the expected calving date.

e-b Means within a row lacking a common superscript letter differ at $P < 0.05$;

Table 5. Least-square means ± SEM for blood metabolic parameters of calves born to dams fed prepartum diets providing 118% (HME), 104% (CME), and 92% (LME) of the predicted metabolizable energy (ME) requirements from day -53 to calving, followed by an ad libitum postpartum lactation diet. Measurements were taken when the calves were 28 days old.

Item	**Experimental treatments**			***P*-value**
	HME	**CME**	**LME**	**Treatment**
Insulin, μg/L	3.48 ± 0.55	4.43 ± 0.55	3.70 ± 0.49	0.47
Glucose, mmol/L	6.54 ± 0.22	6.44 ± 0.22	6.54 ± 0.20	0.92
BHBA[1], μmol/L	66.36 ± 7.86	61.47 ± 7.86	70.0 ± 7.03	0.72
NEFA[2], mmol/L	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.68

[1]BHBA: Beta-hydroxybutyrate; [2]NEFA: Non-esterified fatty acids

Table 6. Least-square means ± SEM for mRNA expression of metabolic energy markers in skeletal muscle of calves born to dams fed prepartum diets providing 118% (HME), 104% (CME), and 92% (LME) of the predicted metabolizable energy (ME) requirements from day -53 to calving, followed by an ad libitum postpartum lactation diet.

Item	**Experimental treatments**			***P*-value**
	HME	**CME**	**LME**	**Treatment**
Glucose metabolism				
IRS1	5.50 ± 1.3	5.98 ± 1.43	4.87 ± 1.13	0.74
SLC2A4	6.28 ± 0.87	6.76 ± 1.01	6.47 ± 0.81	0.92
Myosin heavy chain types				
MYH7	4.18 ± 1.29	2.68 ± 0.83	5.25 ± 1.46	0.07
MYH2a	8.26 ± 1.53 ^{b}	6.46 ± 1.21 ^{ab}	4.51 ± 0.91 ^{a}	0.04
MYH2x	2.59 ± 0.37 ^{b}	3.48 ± 0.47 ^{ab}	4.79 ± 0.59 ^{a}	0.01
MYH1	3.81 ± 0.72 ^{b}	6.60 ± 0.63 ^{a}	3.82 ± 0.63 ^{b}	0.02
Oxidative metabolism				
PPARGC1α	2.41 ± 0.87 ^{b}	6.26 ± 0.87 ^{a}	4.87 ± 0.87 ^{a}	0.04
PPARα	0.84 ± 0.20 ^{b}	1.65 ± 0.38 ^{a}	1.43 ± 0.29 ^{a}	0.04
Fatty acid and lipid metabolism				
PPARγ	2.49 ± 0.30	2.56 ± 0.30	2.53 ± 0.30	0.98
ACACA	0.85 ± 0.16	0.93 ± 0.16	1.16 ± 0.18	0.28
CPT1α	2.66 ± 0.94	3.22 ± 1.10	2.72 ± 0.85	0.80

FASN	1.63 ± 0.62	2.00 ± 0.77	1.66 ± 0.57	0.80
SREBF1	0.98 ± 0.39	1.02 ± 0.38	0.61 ± 0.22	0.19
UCP3	2.38 ± 0.65	2.1 ± 0.48	2.87 ± 0.58	0.45
Muscle mass control				
MEF2A	0.60 ± 0.14 [b]	0.52 ± 0.12 [b]	1.17 ± 0.24 [a]	0.01

IRS1: Insulin receptor 1; SLC2A4: Glucose transporter 4; MYH7: Myosin heavy-chain 7; MYH2a: Myosin heavy-chain 2a; MYH2x: Myosin heavy-chain 2x; MYH1: Myosin heavy-chain 1; PPARGC1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: peroxisome proliferator-activated receptor alpha; PPARγ: peroxisome proliferator-activated receptor gamma; ACACA: Acetyl-CoA carboxylase; CPT1α: Carnitine palmitoyltransferase 1 alpha; FASN: fatty acid synthase; SREBF1: Sterol Regulatory Element-Binding Transcription Factor 1; UCP3: Uncoupling protein 3; MEF2A: myocyte enhancer factor 2A.

[g-b] Means within a row lacking a common superscript letter differ at P < 0.05

FIGURES

Figure 1. Least square means ± SEM of protein abundance of the ratio phospo-AMP-activated protein kinase (p-AMPK)/ AMP-activated protein kinase (AMPK) in skeletal muscle of calves born to beef cows fed 104% (CME), 118% (HME), and 92% (LME) of predicted metabolizable energy intake (ME) at late gestation. Differences were considered when $P \leq 0.05$ and represented by different letters.

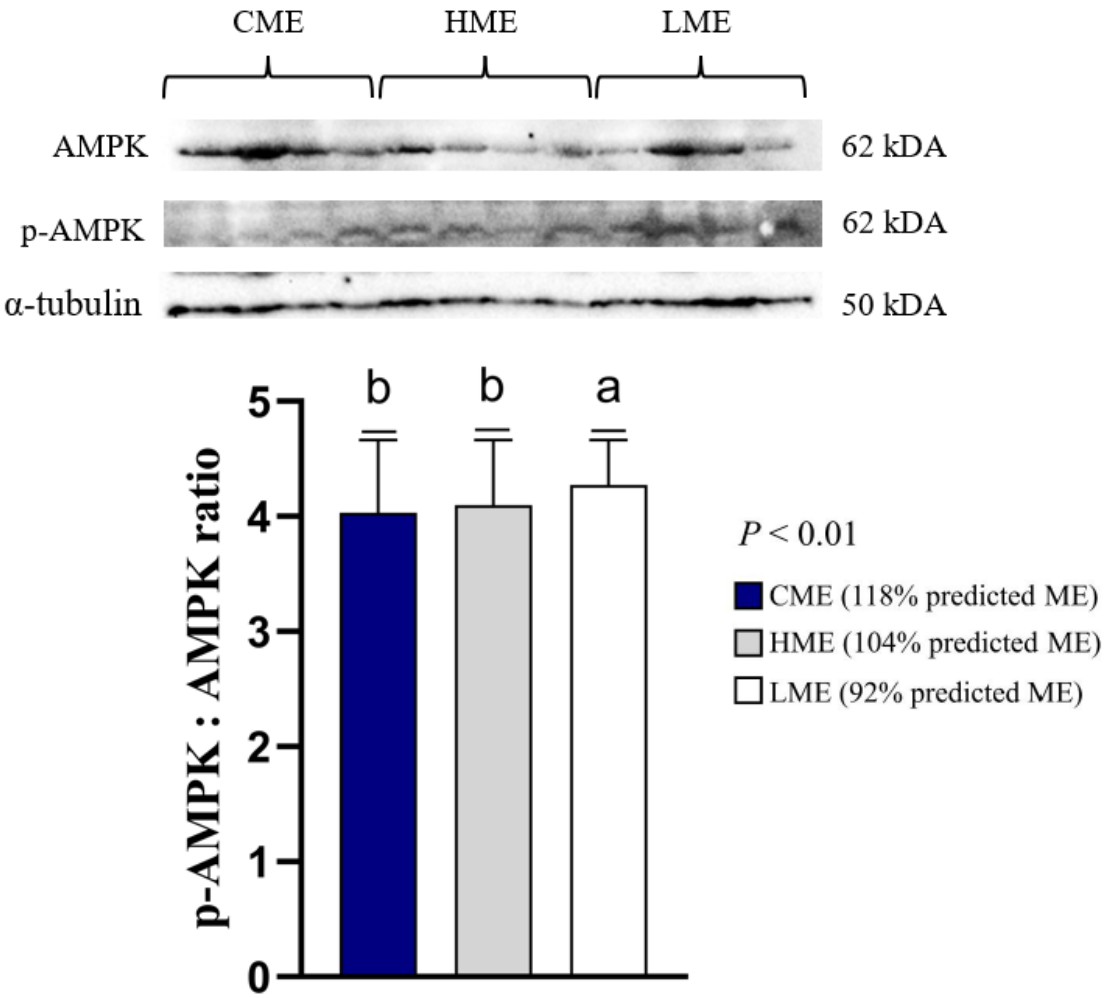
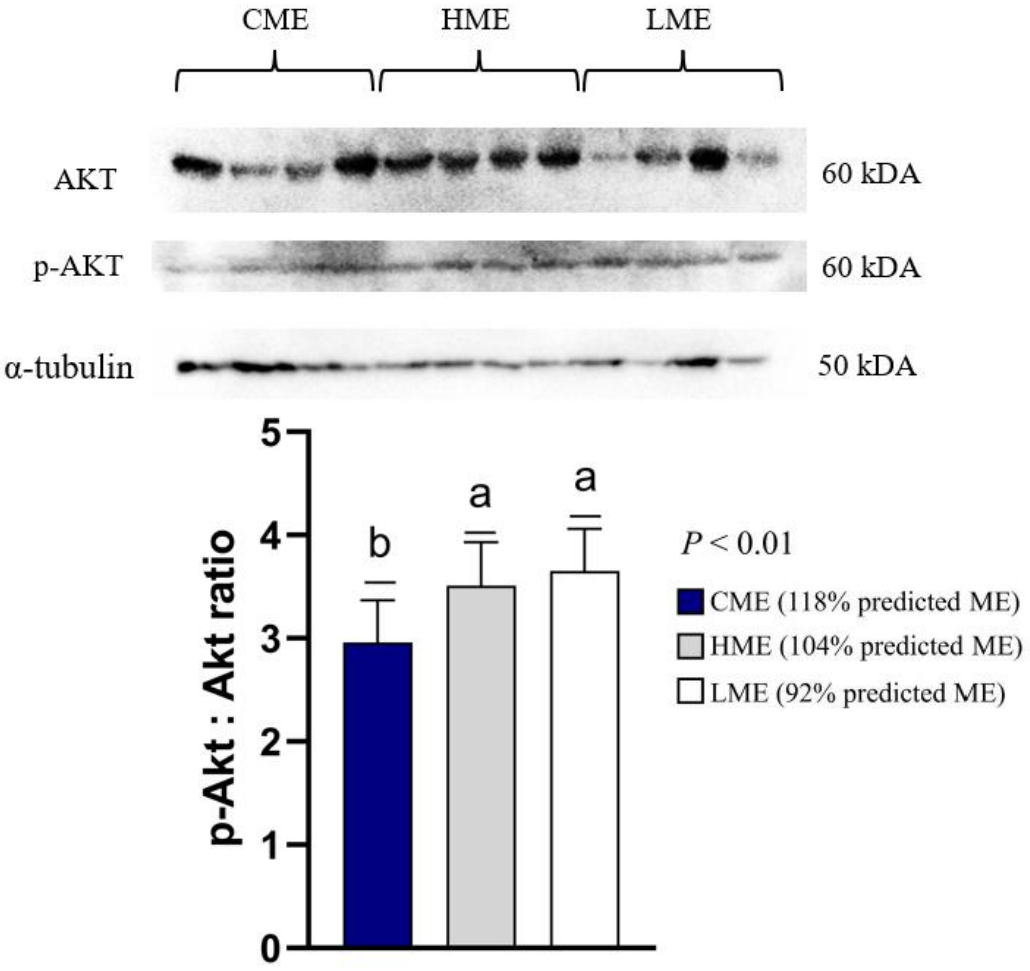


Figure 2. Least square means ± SEM of protein abundance of the ratio phospho-protein kinase B (p-Akt)/ protein kinase B (Akt) in skeletal muscle of calves born to beef cows fed 104% (CME), 118% (HME), and 92% (LME) of predicted metabolizable energy intake (ME) at late gestation. Differences were considered when $P \leq 0.05$ and represented by different letters.



GENERAL CONCLUSIONS

The findings of this study provide new insights into how varying levels of maternal metabolizable energy intake during late gestation can modulate the energy metabolism of skeletal muscle in beef offspring. Although no significant differences were observed in growth performance or blood metabolic profile among treatments, molecular and protein-level adaptations were identified, particularly in calves born to cows fed below the estimated energy requirements. These calves exhibited enhanced AMPK activity, upregulation of genes involved in mitochondrial biogenesis and oxidative metabolism, and a distinct muscle fiber gene expression profile.

Such alterations suggest that the maternal nutritional environment during this critical window may influence the metabolic programming of skeletal muscle, potentially affecting the offspring's capacity for nutrient utilization and metabolic flexibility later in life. These findings underscore the importance of considering fetal metabolic development as a relevant outcome when designing nutritional strategies for pregnant beef cows. Furthermore, they raise the possibility that differences observed in later phases of beef production, such as carcass traits and feed efficiency during finishing, may have origins in the prenatal period, mediated by adaptations in muscle energy metabolism. Therefore, a better understanding of these mechanisms can contribute to more precise and effective nutritional planning aimed at optimizing lifetime productivity in beef systems.