RODRIGO CÍSTOLO LOPES

SUPLEMENTAÇÃO PROTEICA PARA VACAS DE CORTE NO FINAL DA GESTAÇÃ AFETA A EXPRESSÃO GÊNICA NO MUSCULO ESQUELÉTICO MATERNO E AMINOÁCIDOS CIRCULANTES NO PLASMA

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Nutrição e Produção de Ruminantes, para a obtenção do título de Mestre.

Dr. Mateus Pies Gionbelli

Orientador

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RODRIGO CÍSTOLO LOPES

PROTEIN SUPPLEMENTATION IN LATE GESTATION FOR BEEF COWS AFFECTS MATERNAL SKELETAL MUSCLE GENE EXPRESSION AND PLASMA CIRCULATING AMINO ACIDS

SUPLEMENTAÇÃO PROTEICA PARA VACAS DE CORTE NO FINAL DA GESTAÇÃO AFETA A EXPRESSÃO GÊNICA NO MUSCULO ESQUELÉTICO MATERNO E AMINOÁCIDOS CIRCULANTES NO PLASMA

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Aprovado em 03 de Novembro de 2017.

Dr. Daniel Rume Casagrande	UFLA
Dr. Otávio Rodrigues Machado Neto	UNESP/BOTUCATU

Dr. Mateus Pies Gionbelli

Orientador

LAVRAS - MG 2017

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RESUMO

No final da gestação de vacas de corte, as exigências de nutrientes aumentam para atender à taxa de crescimento do concepto e estas matrizes podem alterar o metabolismo no tecido esquelético, hepático e modificar os níveis plasmáticos circulantes de aminoácidos (AA) em função da suplementação de proteína bruta (PB). Quarenta e três vacas Nelore gestantes de fetos machos, [idade média = 6 anos; peso médio = 500 kg] em 193 ± 30 (SD) dias (d) de gestação foram divididas em oito grupos (unidades experimentais, com 4 a 5 vacas cada). Os tratamentos foram 1) Controle (CON, n = 4), dieta baseada em pastagem sem suplementação de proteína bruta (PB) e 2) Suplemento (SUP, n = 4), dieta baseada em pastagem acrescida da suplementação de 2 g / kg de peso corporal de suplemento proteico. Foram coletadas através de biópsia, amostras de fígado e músculo para a análise de expressão relativa de mRNA e amostras de sangue para níveis de AA plasmáticos circulantes. Vacas do grupo CON apresentaram tendência (P = 0.057) para maiores níveis de AA circulantes em comparação ao SUP. Níveis de AA glicogênios foram maiores (p = 0.035) no grupo CON em relação ao SUP. Vacas do grupo CON apresentaram maiores concentrações de Histidina (P = 0.015), Metionina (P = 0.007) e Alanina (P = 0.036) em relação ao grupo SUP. Ambos grupos, CON e SUP não apresentaram diferenças significativas gliconeogênese, transporte de acido graxo e eixo somatotrófico. As vacas CON tenderam a apresentar maiores valores para marcadores relacionados a síntese de tecido muscular esquelético, p7056k (P = 0.060) e GSK3B (P = 0.096). Não foi encontrado diferenças significativas para marcadores relacionados a degradação de tecido muscular esquelético. A suplementação de proteína bruta para vacas de corte no terço final da gestação e mantidas em pastagens altera o perfil de aminoácidos circulantes no plasma e a síntese no tecido muscular esquelético.

Palavras - chave: Expressão de mRNA, metabolismo, nutrição materna, prenhez, proteína bruta, ruminantes

ABSTRACT

In late pregnancy of beef cows, nutrients requirements increase to meet the growth rate of conceptus and dams may alters metabolism in liver and skeletal tissue, and modify the circulating plasma levels of amino acids (AA) in function of crude protein (CP) supplementation. Fourty tree pregnant Nellore cows gestating male fetuses, [average age = 6 years; average weight = 500 kg] at 193 ± 30 (SD) days (d) of gestation were divided into eight groups (experimental units, with 4 to 5 cows each). Treatments were 1) Control (CON, n = 4), pasture-based (PB) diet without crude protein (CP) supplementation and 2) Supplemented (SUP, n = 4), PB diet supplemented with 2 g/kg of body weight of CP supplement. Liver and muscle biopsies for relative mRNA expression, and circulating plasma levels of AA were recorded. The CON-fed cows tended to have greater (P = 0.057) total circulating AA than SUP-fed cows. Circulating glycogenic AA was greater (p = 0.035) in CON than in SUP cows. CON cows was greater for Histidine (P = 0.015), Methionine (P = 0.007) and Alanine (P = 0.036) than SUP cows. CON and SUP-fed shown no significant differences for gluconeogenesis, fatty acid transport and signaling axis. CON cows tended to be greater than SUP cows in mRNA expression of markers related to Skeletal muscle synthesis, p7056k (P = 0.060) and GSK3B (P = 0.096). No significant differences were found for mRNA expression of markers related to Skeletal muscle degradation. Crude protein supplementation for late pregnant beef cows in a pasture-based diet change the profile of plasma circulating AA and synthesis of skeletal muscle tissue.

Keywords: Crude protein, maternal nutrition, metabolism, mRNA expression, pregnancy, ruminants

INTRODUÇÃO

Alguns modelos de padrões metabólicos para gestação foram desenvolvidos para ovelhas e vacas (Bell, 1995b; Bell et al., 1995). De acordo com Bell et al. (2005), é esperado que durante o terço final de gestação, 35 a 40% da energia fetal é fornecida como glicose e lactato e cerca de 55% é fornecida como aminoácidos. A maioria dos 5 a 10% restantes é fornecida por acetato, o que é muito pouco, em relação à sua relativa abundância e importância para o sistema materno.

. A capacidade da placenta em transportar ácidos graxos não esterificados de cadeia longa (AGNE) e corpos cetônicos é bastante limitada (Bell *et al*., 2005), negando o acesso fetal a substratos derivados da mobilização de gordura materna e, assim, restringindo a capacidade do feto de crescer à custa de reservas maternais de energia

Em ovelhas bem alimentadas, no terço final da gestação, a captação de glicose uterina corresponde a cerca de 30 a 50% do aporte de glicose materna (Bell et al., 2005), levando-se em consideração também o incremento da exigência de glicose da matriz necessário para suportar a gestação (Freetly and Ferrell, 1998). Não são disponíveis estudos sobre o parcionamento direto de aminoácidos entre o útero grávido e os tecidos maternos não uterinos (Bell et al., 2005), no entanto, modelos múltiplos animais de restrição de crescimento uterino tem sido utilizados para estudar concentração de amino ácidos e fluxo útero placental (Vonnahme et al., 2015).

Cabe ressaltar que essas estimativas são para ovelhas consumindo entre 110 e 140% das exigências de proteína bruta para gestação, e que em ovelhas consumindo apenas o nível correspondente às exigências diárias de proteína, a estimativa é de que a disponibilidade de aminoácidos circulantes é aumentada pela mobilização de proteína dos tecidos da carcaça materna (principalmente músculo esquelético) em cerca de 10% (Bell et al., 1998), com a quantidade residual sendo utilizada como suporte metabólico, glândulas mamárias e vísceras maternas (Bell and Ehrhardt, 2000). Em ovelhas prenhes alimentadas com quantidades de proteínas próximas ou abaixo da recomendação dos sistemas nutricionais, estima-se que a disponibilidade de aminoácidos circulantes seja aumentada aproximadamente dez unidades percentuais por mobilização protéica do musculo esquelético materno (Bell and Ehrhardt, 2000).

A gliconeogênese hepática aumenta em ovelhas durante o final da gestação, mesmo quando o consumo de alimento não é aumentado acima dos padrões de animais

não gestantes (Freetly and Ferrell, 1998). Parte desse aumento na gliconeogênese é suportado pelo aumento da captação hepática de lactato (Freetly and Ferrell, 1998), aparentemente derivado do metabolismo e aumento da glicose útero-placentária materna em tecidos periféricos (Bell and Ehrhardt, 2000). Uma parte ainda é suportada pelo aumento da captação hepática de glicerol, especialmente se a mobilização de gordura é aumentada com a aproximação do parto (Freetly and Ferrell, 2000). Aminoácidos mobilizados a partir de tecidos da carcaça materna (McNeill et al., 1997) também podem ajudar a sustentar uma taxa maior de gliconeogênese hepática no terço final da gestação.

Os mesmos últimos autores evidenciaram uma concentração crescente no hormônio do crescimento plasmático e um declínio abrupto no IGF-I e na insulina, iniciando duas semanas antes do parto em vacas. Em contraste com as matrizes que apresentaram baixa escore de condição corporal (ECC), aquelas que pariram com ECC médio e alto, a expressão de genes envolvidos no eixo de sinalização de hormônio de crescimento / IGF-I (GHRIA e IGF-I) foi menor (Akbar et al., 2015), indicando uma provável menor degradação da via de sinalização muscular, alta produção de leite e maior gliconeogênese nestes animais.

A taxa de síntese de proteína hepática aumentou em 45% no final da gestação em vacas leiteiras, mesmo quando o consumo de matéria seca e proteína bruta estava em declínio (Bell, 1995a). Essa informação é consistente com um aumento de deposição de proteína hepática observado por McNeill et al. (1997) e uma diminuição na deaminação hepática de aminoácidos no final da gestação de ovelhas (Freetly and Ferrell, 1998). Significativa perda de nitrogênio nos tecidos da carcaça de ovelhas no terço final da gestação foi observada (McNeill et al., 1997), possivelmente em função da mobilização de aminoácidos do músculo esquelético. Tem sido observado, no entanto, que não existem estudos que avaliem conjuntamente a absorção de nutrientes e o metabolismo dos tecidos hepático, adiposo e esquelético de vacas no final da gestação.

Em sistemas de produção onde bovinos de corte são criados em regiões tropicais, as matrizes são muitas vezes programadas para parirem no início da estação chuvosa, quando a disponibilidade de pastagens de qualidade são altas (Duarte et al., 2013). Como conseqüência, essas vacas passam a segunda metade da gestação na época do ano com menor disponibilidade e qualidade de forragem. Nesses casos, os níveis de proteína bruta de pastagem tropical são geralmente entre 4 e 6%, apresentando alta concentração

de fibra insolúvel e lignina, e baixos conteúdos de compostos nitrogenados. (Detmann et al., 2009).

Assim, baseado na alta demanda de aminoácidos durante o fim da gestação, hipotetizamos que os níveis de aminoácidos circulantes, metabolismo hepático e no músculo esquelético em vacas de corte mantidas em pastagem de baixa qualidade será drasticamente alterado quando estas recebem suplementação proteica. No entanto, nossa hipótese principal é que vacas gestantes suplementadas com proteina bruta no terço final de gestação aumenta a abundância de marcadores para síntese proteica, gliconeogenese, eixo somatotrópico e síntese e transporte de ácido graxo no fígado. Além disso, diminuição da abundância de marcadores para degradação proteica. Também, vacas gestantes mantidas em pastagem sem suplementação proteica durante a estação seca, mobiliza tecido muscular esquelético para suprir a demanda por glicose e aminoácidos do feto em crescimento.

O objetivo principal deste estudo foi avaliar se a suplementação no terço final de gestação de vacas de corte influencia positivamente a abundância de fatores de transcrição no fígado e músculo esquelético. Nós também objetivamos identificar possíveis mudanças nos níveis de aminoácidos circulantes de vacas de corte do final da gestação em função da suplementação proteica.

REFERÊNCIAS

Akbar, H. et al. 2015. Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. J. Dairy. Sci. 98: 1019-1032.

Bell, A., and R. Ehrhardt. 2000. 16 Regulation of Macronutrient Partitioning between Maternal and Conceptus Tissues in the Pregnant Ruminant. RUMINANT PHYSIOLOGY: 275.

Bell, A., R. Ehrhardt, W. Hansel, G. Bray, and H. Donna. 1998. Placental regulation of nutrient partitioning during pregnancy. In: Nutrition and reproduction. p 229-254.

Bell, A. W. 1995a. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73: 2804-2819.

Bell, A. W. 1995b. Use of ruminants to study regulation of nutrient partitioning during pregnancy and lactation. In: Symposium on the Animal Model for Human Research, Ottawa, Canada. p 41-62.

Bell, A. W., C. L. Ferrell, and H. C. Freetly. 2005. Pregnancy and Fetal Metabolism. In: J. Dijkstra, J. M. Forbes and J. France (eds.) Quantitative aspects of ruminant digestion and metabolism No. 1. p 523-550. CAB International, Oxfordshire.

Bell, A. W., R. Slepetis, and R. A. Enrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy. Sci. 78: 1954-1961.

Detmann, E. et al. 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis–Menten kinetics. Livestock Science 126: 136-146.

Duarte, M. S. et al. 2013. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. Livestock Science 153: 60-65.

Freetly, H. C., and C. L. Ferrell. 1998. Net flux of glucose, lactate, volatile fatty acids, and nitrogen metabolites across the portal-drained viscera and liver of pregnant ewes. J. Anim. Sci. 76: 3133-3145.

Freetly, H. C., and C. L. Ferrell. 2000. Net flux of nonesterified fatty acids, cholesterol, triacylglycerol, and glycerol across the portal-drained viscera and liver of pregnant ewes. J. Anim. Sci. 78: 1380-1388.

McNeill, D. M., R. Slepetis, R. A. Ehrhardt, D. M. Smith, and A. W. Bell. 1997. Protein requirements of sheep in late pregnancy: partitioning of nitrogen between gravid uterus and maternal tissues. J. Anim. Sci. 75: 809-816.

Vonnahme, K. A., C. O. Lemley, J. S. Caton, and A. M. Meyer. 2015. Impacts of maternal nutrition on vascularity of nutrient transferring tissues during gestation and lactation. Nutrients 7: 3497-3523.

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Protein supplementation in late gestation for beef cows affects maternal skeletal muscle gene expression and plasma circulating amino acids

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SUMMARY

In late pregnancy of beef cows, nutrients requirements increase to meet the growth rate of conceptus and dams may alters metabolism in liver and skeletal tissue, and modify the circulating plasma levels of amino acids (AA) in function of crude protein (CP) supplementation. Fourty tree pregnant Nellore cows gestating male fetuses, [average age = 6 years; average weight = 500 kg] at 193 ± 30 (SD) days (d) of gestation were divided into eight groups (experimental units, with 4 to 5 cows each). Treatments were 1) Control (CON, n = 4), pasture-based (PB) diet without crude protein (CP) supplementation and 2) Supplemented (SUP, n = 4), PB diet supplemented with 2 g/kg of body weight of CP supplement. Liver and muscle biopsies for relative mRNA expression, and circulating plasma levels of AA were recorded. The CON-fed cows tended to have greater (P = 0.057) total circulating AA than SUP-fed cows. Circulating glycogenic AA was greater (p = 0.035) in CON than in SUP cows. CON cows was greater for Histidine (P = 0.015), Methionine (P = 0.007) and Alanine (P = 0.036) than SUP cows. CON and SUP-fed shown no significant differences for gluconeogenesis, fatty acid transport and signaling axis. CON cows tended to be greater than SUP cows in mRNA expression of markers related to Skeletal muscle synthesis, p7056k (P = 0.060) and GSK3B (P = 0.096). No significant differences were found for mRNA expression of markers related to Skeletal muscle degradation. Crude protein supplementation for late pregnant beef cows in a pasture-based diet change the profile of plasma circulating AA and synthesis of skeletal muscle tissue.

Keywords: Crude protein, maternal nutrition, metabolism, mRNA expression, pregnancy, ruminants.

INTRODUCTION

Models for metabolic patterns of gestation and lactation have been developed for ewes and cows (Bell, 1995; Bell et al., 1995). According to Bell et al. (2005), it is expected that during the last third of pregnancy, glucose and lactate supply 35-40% of placental energy demand and 55% is provided by amino acids. The remaining 5-10% is provided by acetate, which is very small in comparison to its relative abundance and importance to the maternal system. The placenta capacity to transport non-esterified fatty acids (NEFA) and ketone bodies is limited (Bell *et al*., 2005), limiting the capacity of fetal growth based on maternal energy reserves.

Effects of pregnancy on the quantitative metabolism of amino acids have not been studied quantitativelly in ruminants (Bell et al., 2005), therefore, multiple animal models of intrauterine growth restriction have been used to examine amino acid concentrations and flux across the uteroplacenta (Vonnahme et al., 2015; Mordhorst et al., 2017). In pregnant ewes fed amounts of protein near or below the recommendation of the nutritional systems, it is estimated that the availability of circulating amino acids is increased approximately ten percent units by protein mobilization of dam skeletal muscle (Bell and Ehrhardt, 2000). Amino acids mobilized from maternal carcass tissue may also support a higher rate of hepatic gluconeogenesis in late gestation of ruminants (McNeill et al., 1997).

The mechanisms regulating protein mobilization in late gestation of cows are still not clear. Nevertheless, the pattern of reduced protein synthesis, and possible proteolysis in maternal carcass tissue is consistent with observed changes in plasma concentrations to key endocrine regulators of tissue protein metabolism, including insulin and insulin-like growth factor IGF-I (Bell et al., 2000). Significant loss of nitrogen in ewe's carcass tissue in late third of pregnancy was observed (McNeill et al., 1997), possibly due to amino acids mobilization in skeletal carcass tissue. It has been observed, however, that there are no studies jointly evaluating nutrient uptake and tissue metabolism in liver, adipose and skeletal muscle tissue of beef cows in late pregnancy.

Thus, based on the high demand for amino acids during late gestation, we hypothesized that the circulating plasma levels of amino acids and, liver and skeletal muscle metabolism of beef cows are drastically changed when cows kept in a low quality pasture-based system are supplemented with crude protein in late gestation. This

study aimed to evaluate if the CP supplementation in late gestation beef cows influences positively the abundance of transcript factors in liver and skeletal muscle. We also aim to identify possible changes in the plasma level of circulating amino acids in late pregnant beef cows as a function of crude protein supplementation.

MATERIALS AND METHODS

The experiment was carried out at the Beef Cattle Facilities of the Universidade Federal de Viçosa (UFV), Viçosa, State of Minas Gerais, Brazil. Animal welfare and procedures were approved by the Ethics Committee on Animal Use of UFV. Animals and reproductive management prior to the beginning of the experiment has been already reported by Trece (2017).

Experimental design and Management

Forty tree pregnant Nellore cows gestating male fetuses, [average age = 6 years; average weight = 500 kg] at 193 ± 30 (SD) d of gestation were divided into eight groups (experimental units, with 4 to 5 cows each). At the beginning of the experimental period the animals were treated for internal and external parasites (Ivomec®, Paulínia, Brazil). The eight groups were divided at random into two treatments (control and supplemented) being four experimental units to each treatment. Experimental area was composed of eight 4.0-ha paddocks predominantly composed by Brachiaria decumbens (Syn.Urochloa). The groups were transferred throughout different paddocks every week to eliminate the effect of the pasture conditions among paddocks, reducing the grazing and relief effects, also proximity of supplement and water troughs. Animals had permanent access to clean water and covered feeding troughs for supplement intake.

The experiment was carried out in a completely randomized design with two treatments, being: 1) Control (CON, n = 4) treatment with cows in a pasture-based diet without crude protein (CP) supplementation and 2) Supplemented (SUP, n = 4) treatment, with cows in the pasture-based diet supplemented at the level of 2 g/kg of protein supplement. The supplement was offered every day at 11:00 a.m. from 193th day of gestation until calving, which was consisted of a 50:50 mixture (Table 1), of soybean meal and commercial supplement (assurance levels per kilogram of product: 70 g Calcium (max); 50 g Calcium (min); 15 mg Cobalt (min); 255 mg Copper (min); 15 g Sulfur (min); 2000 mg Fluorine (max); 20 g Phosphorus (min); 15 mg Iodine (min); 510

mg Manganese (min); 340 NPN protein eq. (max); 450 g CP (min); 4 mg Selenium (min); 95 g Sodium (min); 850 mg Zinc (min); 50 mg Flavomycin).

During the trial period dams were weighed without feed or water restriction every 28 days at 6:00 a.m. to adjust supplement offered. The amount of supplement offered to each group was then adjusted every 28 days based on the cows body weight. Body weight adjusted to parturition (adBWP) and concept average daily gain (conADG) were calculated according Gionbelli et al. (2015).

Body condition scores (BCS) were assessed on a scale ranging from 1 (severely emaciated) to 9 (very obese) Nicholson and Butterworth (1986) with 0.5 partial scoring and was determined by observation and palpation. Body condition scores assessment were performed at the beginning (193d of gestation) and pre-calving (280d of gestation**)** period by three trained evaluators in a double-blind scheme. Each evaluator did not know the result of the evaluation of the other, and the average score within evaluators was calculated.

Pasture and total dry matter intake

Pasture and supplement intake of cows were estimated using internal and external markers. Briefly, chromium oxide was used to estimate total fecal excretion (dry matter basis), indigestible NDF (iNDF) was used to estimate the total dry matter intake and supplement intake was estimated by adding titanium dioxide to the supplement. Fecal samples were collected from each cow during 4 consecutive days (at 1600, 1400, 0900, 0600 h for the first to four d, respectively) from 267 to 270 days of gestation. Fecal samples (60 g of wet weight from each day of collection) were pooled for each animal and stored at -20ºC for further analyses. At fifth trial day, forage samples were collected in each paddock by hand-plucking method and were used to estimate the forage intake. Forage samples were also collected by hand-plucking and the average forage composition is shown on Table 1.

All analyses such as, crude protein (CP), neutral detergent fiber corrected to ash and protein (apNDF), acid detergent fiber corrected to ash and protein (apADF), indigestible fiber detergent neutral (iNDF), lignine (LIG), forage mass, potentially digestible dry matter (pdDM) were described by Detmann et al. (2012).

Skeletal muscle and liver tissue sampling

Tissue biopsy samples were obtained from each animal at 265 days of gestation. The muscle tissue biopsies were performed on the left side of the cows in Longissimus dorsi muscle. The respective procedure will be detailed soon after.

1- Clip and scrub a 15x15 cm area of skin of the cow with 70% alcohol; 2- Inject 15 ml of a local anesthetic (2% lidocaine); 3- After 10 minutes, performing a 10 cm incision; 4- Remove a 3 cm sample of both tissues using forceps and a scalpel; 5- Apply at the incision site an antibiotic-based benzylpenicillin and streptomycin, spray with antiseptic, saturate and treat with larvicide; 6- Suture an area; 7- To prevent any post-surgical infection, inject anti-inflammatory and antibiotic intramuscularly; 8- Daily monitor the healing process; 9- Remove the sutures after two weeks.

All muscle samples were immediately placed in microcentrifuge tubes, snap-frozen in liquid nitrogen, and stored at –80°C until analysis.

The site of liver biopsy was on the right rib cage at the 11th intercostal space. The respective procedure will be detailed soon after.

1- Clip and scrub a 5x5 cm area of skin of the cow with 70% alcohol; 2- Inject 10 ml of a local anesthetic (2% lidocaine); 3- After 10 minutes, performing a 0.5 cm incision; 4- Introduce the needle type Tru-Cut 20 mm diameter and collect the sample; 5- Apply a larvicide at the site of incision; 6- Suture an area; 7- To prevent any post-surgical infection, inject anti-inflammatory and antibiotic intramuscularly; 8- Daily monitor the healing process; 9- Remove the sutures after two weeks;

All liver samples were immediately placed in microcentrifuge tubes, snap-frozen in liquid nitrogen, and stored at –80°C until analysis.

Amino acid circulating levels

At 280 days of gestation (10 d prior to parturition), at 6:00 am, blood samples (1x10 mL) were collected by coccygeal venipuncture into evacuated tubes containing EDTA to prevent blood coagulation. Whole blood were centrifuged at 3,000 × g at 4°C for 12 min and plasma was harvested, aliquoted into tubes, and carried to a commercial laboratory to subsequent analysis. Plasma samples were analyzed by VIÇOSA LAB laboratory (Viçosa, MG - Brazil) for circulating amino acids (Aspartic Acid, Glutamic acid, Asparagine, Histidine, Serine, Glutamine, Arginine, Tyrosine, Alanine,

Triptophan, Methionine, Valine, Phenylalanine, Isoleucine, Leucine) using the High-Performance Liquid Chromatography (HPLC) method. In which each amino acid was analyzed individually and grouped according its function. Essentials: Arginine, Phenylalanine, Histidine, Isoleucine, Methionine, Leucine, Triptophan, Valine; Nonessential: Aspartic Acid, Glutamic acid, Alanine, Asparagine, Serine, Glutamine, Tyrosine; Branched-Chain AA: Leucine, Valine, Isoleucine. Glucogenic: Methionine, Valine, Arginine, Histidine, Glutamine, Glutamic acid, Aspartic Acid, Asparagine, Serine, Alanine; Ketogenic: Leucine; Glucogenic and Ketogenic: Isoleucine, Phenylalanine, Tyrosine, Triptophan.

Gene Expression

The following genes were studied: PCK1, phosphoenolpyruvate carboxykinase 1; PC, pyruvate carboxylase; GHR1A, growth hormone receptor 1ª; IGF1, insulin-like growth factor 1; ACACA, acetyl-CoA carboxylase alpha; GSK3B, glycogen synthase kinase 3β; eIf4E, eukaryotic translation initiation factor 4E; MuRF1, muscle ring finger 1; p7056k, ribosomal protein S6 kinase.

Sequences published in the GenBank, a National Center for Biotechnology Information (NCBI) platform, were used to design primers for the reference and target genes. The primers (Table 2) were designed using the OligoPerfect Designer software (Life Technologies, Grand Island, USA). The PCR primers were commercially synthesized (Life Technologies, São Paulo, BR) and reconstituted to a final concentration of 10 µmol.

Total RNA was extracted from liver samples using QIAzol (QIAGEN, Valencia, CA) and treated with DNA-free DNase (Ambion, Austin, TX) according to the manufacturer's instructions. To analyse the 28S and 18S rRNA bands, the total RNA was electrophoresed in a 1.0% (m/v) agarose gel, stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA) and visualized with a UVItec FireReader XS D-77Ls20M (UVItec, Cambridge, UK). The RNA quantity (ng/µL) and quality (260/280 and 260/230) were assessed using a spectrophotometer (NanoDrop Spectrophotometer ND 1000, Thermo Scientific, Wilmington, DE) at 260 nm. cDNA synthesis was performed using the HighCapacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions, and samples were stored at −20°C. An ABI PRISM 7500 Real-Time PCR system (Applied

198 Biosystems) was used with a SYBR Green detection system (Applied Biosystems) for
199 quantitative gene expression analysis by reverse-transcription quantitative PCR (RT-
200 qPCR). The RTqPCR programme used was as follows: 50°C for 2 min, 95°C for 10
201 min, 40 cycles of 95°C for 15 sec, 60°C for 1 min, and 95°C for 15 sec. The data were
202 collected and stored using Os 7500 Fast Software (Version 2.1; Applied Biosystems).
203 For each reaction, 1.0 µL of cDNA (10 ng/µL), 0.3 µL of each primer (1.5 µM; forward
204 and reverse) and 5.0 µL of SYBR Green Master Mix were combined in a 10.0-µL final
205 volume per sample in a 96-well MicroAmp Optical plate (Applied Biosystems).

206 The RT-qPCR analyses of each studied gene were performed using cDNA from
207 11 biological replicates, with 2 technical replicates per biological replicate. The results
208 were normalized using the threshold cycle (CT) method for the expression of the
209 reference genes 18S (18S ribosomal) and glyceraldehyde-3-phosphate dehydrogenase
210 (GAPDH). The CT was determined by the total number of cycles using the comparative
211 CT method. A validation assay was performed to demonstrate that the amplification
212 efficiencies of the target and reference genes were approximately equivalent. Standard
213 curves were generated for the studied genes with the following dilutions: 1:5, 1:25,
214 1:125, 1:625 and 1:3125. The relative expression of target genes was obtained by the $2^{-\Delta\Delta Ct}$
215 method (Livak and Schmittgen, 2001).

216 **Statistical Analysis**

217 Data were analyzed through a model including the fixed effect of nutritional
218 treatment, as follows:

219 $$Y_{ij} = \mu + NT_{i} + e_{ij}$$

220 where μ is the overall mean, NT_i is the *i*th level of the fixed effect of nutritional
221 treatment and e_{ij} is the random error associated with $Y_{ij.}$

222 Gene expression levels were transformed using the natural logarithm of the
223 expression values +1 to achieve normality. Outliers were removed to achieve normality
224 using Shapiro-Wilks test at $\alpha = 0.05$. Least square means were estimated for treatment
225 effect and compared using T test at $\alpha = 0.05$. All statistical procedures were performed
226 using the MIXED procedure from SAS version 9.2 (SAS Inst., Inc., Cary, NC).

227

RESULTS

Intake, average daily gain and body condition score

Dry matter intake of forage was similar for CON and SUP (P = 0.202; 5.97 vs. 5.36 ± 0.296 kg [SEM]), but when expressed as g/kg of BW, dry matter intake of forage was greater (P = 0.03) in CON than SUP cows (11.11 vs. 9.49 ± 0.389 g/kg of BW). Total dry matter intake was similar for CON than SUP (P = 0.455; 5.97 vs. 6.30 ± 0.296 kg) indicating forage substitution by supplement (substitution effect). Crude protein intake was greater for SUP vs. CON (P = 0.05; 0.673 vs. 0.331 ± 0.026 kg). A detailed evaluation of feed intake and digestibility was shown by Trece (2017).

There were no differences in body weight adjusted at parturition (P = 0.160), average daily gain (P = 0.450), concept average daily gain (P = 0.548) and body condition score (P = 0.542) for CON vs. SUP cows (Table 3). Also no differences are found in birth weight (P = 0.649) of calves from these dams. Thus, it can be noted that the effects of pregnant cows' supplementation in late gestation on amino acids circulating levels and muscle and liver tissue expression occurred independently of cows' average daily gain and calf size.

Circulating levels of amino acids

The CON-fed cows tended to have greater (P = 0.057) total circulating amino acids than SUP-fed cows. In an attempt to better understand the metabolic processes in which amino acids participate, amino acids were grouped according to their functions (Table 4). The level of circulating glycogenic amino acids was greater (p = 0.035) in CON than in SUP cows, but no significant differences (P = 0.254) were found for circulating level of ketogenic amino acids among treatments. There was a tendency (P = 0.084) for nonessential amino acids be greater in CON than in SUP cows, however for essential amino acids no significant (P = 0.146) differences were detected in both treatments. As well, for glycogenic/ketogenic (P = 0.180) and branched chain (P = 0.250) amino acids, no significant difference was detected. The amino acids have been individually shown with its respective significance in Table 4. We did not find significant differences for Arginine (P = 0.223), Phenylalanine (P = 0.268), Isoleucine (P = 0.120), Leucine (P = 0.254), Tryptophan (P = 0.789), Valine (P = 0.360), Aspartate (P = 0.988), Glutamate (P = 0.460), Glutamine (P = 0.102), Tyrosine (P =

0.231). CON cows shown a tendency to be greater than SUP cows for Asparagine (P = 0.052) and Serine (P = 0.066), in addition CON cows were greater for Histidine (P = 0.015), Methionine (P = 0.007) and Alanine (P = 0.036) circulating levels than SUP cows. In an attempt to better understand these data, bars graphic are shown (Figure 1) for total, glucogenic and nonessential, and individual circulating plasma amino acids as methionine, histidine, asparagine, serine and alanine.

Skeletal muscle and liver tissue gene expression

The CON and SUP-fed cows did not have significant differences in liver tissue mRNA expression (Table 5) for gluconeogenesis represented for the following markers, PCK1 (P = 0.598) and PC (P = 0.690). The mRNA expression of markers GHR1A (P = 0.993) and IGF1 (P = 0.975) approaching signaling axis also did not shown significant difference. No significant differences were found in mRNA expression for fatty acids transport in liver represented for ACACA (P = 0.872).

In addition, we found a tendency to SUP cows have been greater than CON cows in mRNA expression of markers related to Skeletal muscle synthesis (Figure 2), which are p7056k (P = 0.060) and GSK3B (P = 0.096). In effort to study the same mechanism in skeletal muscle tissue, no significant differences were found for mRNA expression of eIF4E (P = 0.253).

No significant differences were found with respect to Skeletal muscle degradation, whose were studied with mRNA expression of markers MuRF1 (P = 0.153) and Antrogin (P = 0.424).

DISCUSSION

Although dry matter intake of forage was similar among treatments, the CP intake was greater for SUP cows, due to the supplementation, achieving the effectiveness of treatment application in this trial. In beef cattle production systems raised in tropical regions, cows are often programmed to bred at the beginning of the rainy season, when availability of quality pasture are high (Duarte et al., 2013). As a consequence these cows spend the second half of pregnancy at the time of the year with lower availability and quality of feed. In such cases, tropical pasture crude protein levels are generally between 4 and 6% of dry mater, presenting high concentration of insoluble fiber and lignin, and low nitrogenous compounds contents (Detmann et al., 2009).

In an attempt to compensate this deficit of protein, CON cows had a major intake of forage when expressed as g/kg of BW, although the total dry matter intake of both treatments was similar due to the effect of forage intake substitution by the supplement. According to Wood et al. (2013) pregnant cows may metabolize energy reserves and alter their metabolism to meet the energetic demands of the growing fetus, without changing dry matter intake or general growth, an example of these mechanism are increase in circulating Non-sterificad fatty acids (NEFA), Beta hydroxybutirate (BHBA) and urea, and decreasing total cholesterol, accompanied by reduced kidney fat weight. Data from Trece (2017) report no differences among SUP vs. CON cows for NEFA ($p = 0.911$; 0.55 vs. 0.56 ± 0.043) and BHBA ($p = 0.476$; 0.29 vs. 0.25 ± 0.041). As reported by Mordhorst et al. (2017) despite SUP cows were fed with a CP supplement, possibly cows of both group (SUP and CON) were not in a metabolic state different.

Body condition score of SUP and CON cows were similar, this is not agree with previous studies whom late gestation cows consuming low quality forage were supplement (Stalker et al., 2007; Winterholler et al., 2012; Bohnert et al., 2013). Both group of cows shown a reduction in BW during the trial (cow ADG – concept ADG), which SUP cows lost -0.295 kg/d and CON cows lost -0.404 Kg/d. According to Crookenden et al. (2015) increasing metabolizable energy (ME) intake up to 5.0 kg DM/cow above requirements for maintenance, pregnancy, and actively during the dry season occurs in molecular mechanism of BW and BCS gain, that may be explained by expression of transcript abundance in liver and adipose tissue.

Regarding to energetic metabolism of ruminants, starch molecules are fermented in volatile fatty acid and the animal becomes largely dependent on hepatic gluconeogenesis for the supply of glucose. Propionate is the primary precursor for hepatic gluconeogenesis in ruminants, which the generated concentration is directly related to dietary intake of starch substrates that can be fermented by amylolytic bacteria in the rumen (Drackley et al., 2001). In an absence of propionate, L-lactate, glycerol or amino acids may contribute for liver gluconeogenesis.

In our study, total amino acids tended to be greater for CON cows, this data agree with (Bell and Ehrhardt, 2000) when they affirm that in pregnant ewes ingesting amounts of protein near or below the recommendation of the nutritional systems, it is

estimated that the availability of circulating amino acids is increased approximately ten percent units by protein mobilization of dam skeletal muscle. Our results show a difference of twelve percent above for CON than SUP cows. This elevated concentration in blood amino acid may be occurred due to a compensation mechanism by the placenta for their uptake with increased transport capacity (Mordhorst et al., 2017).

Greater circulating levels of glucogenic AA were observed in for CON than in SUP cows, although the mRNA expression markers for liver gluconeogenesis did not shown significant differences among treatments we expected that in both groups the liver uptake for gluconeogenesis was by dietary substrates and amino acid mobilization. As CON cows had a lack in dietary substrates, they likely needed to alther their metabolism to meet the nutrients demands of the growing fetus (Wood et al., 2013), indicating a major level of circulating glucogenic amino acids for this group and no significant differences in birth weight for calves from SUP and CON dams.

We found a tendency to SUP cows to be greater in skeletal protein synthesis thought the mRNA expression of markers p7056k and GSK3B and no significant differences were found for skeletal muscle degradation for these cows. Note that in both groups cows presented weight looses, however this loss occurred more pronounced in CON cows, because the skeletal muscular synthesis was lower in them. A protein turnover is a balance among protein synthesis and breakdown, thus these date indicate that protein breakdown was similar among treatments and protein synthesis was slightly more pronounced in SUP cows, nevertheless indicating a negative balance in the two groups, observed by weight loss even in CON and SUP cows.

Methionine, glycine, serine, and histidine actively participate in one-carbon metabolism pathway and, therefore, the methylation of proteins and DNA, thus regulating gene expression and the biological activity of proteins (Wu, 2009). . When referring of transition period in dairy cows, methionine, histidine and cysteine may be a limiting AA (Zhou et al., 2016), they observed that the concentrations of these AA did not return to prepartal levels by 28 days postpartum. Particularly, methionine is the precursor of sulfur-containing AA including glutathione and taurine, two intracellular antioxidants (Kalhan and Marczewski, 2012), to immune system works well a great concentration of glutathione is necessary.

Alanine inhibits pyruvate kinase and then regulates gluconeogenesis and glycolysis to assure the production of liquid glucose by hepatocytes during periods of feed restriction (Meijer, 2003). In this study we evaluated the gene expression of enzymes (PC and PCK1) related to the conversion of pyruvate to phosphoenolpyruvate via the gluconeogenesis pathway. Parallel to it, there is the glycolysis pathway, which converts phosphoenolpyruvate to pyruvate through the enzyme pyruvate kinase (PK). Unfortunately, this last enzyme was not evaluated in this trial.

A crude protein supplementation and supplement amount did not vary transcript abundance of the hepatic gluconeogenic genes PCK1 and PC. Gene Transcription involved in gluconeogenesis was expected to increase, as increased the efficiency of nutrient utilization, thus increasing hepatic gluconeogenesis. A possible reason for the lack of effect is that with a crude protein supplementation we may improve a synchronization of energy and protein supply thereafter a microbial protein synthesis and nutrient utilization, however a poor quality and quantity of forage made it impossible to happen. With a starch-based supplement we could increase ruminal propionate production and thus intensify hepatic gluconeogenesis (Doelman et al., 2012). To our knowledge, we were expecting a greater abundance of PCK1, PC, ACACA, GHR1A and IGF-1 in liver for SUP cows, however the supplemental diet was not capable to make it. Further research is required to determine the specific reasons for these responses.

In conclusion, a crude protein supplementation for late pregnant beef cows in a pasture-based diet change the profile of plasma circulating amino acids and synthesis of skeletal muscle tissue. Although it is not able to promote significant difference in calf birth weight and to prevent dams from weight loss.

REFERENCES

Bell, A., and R. Ehrhardt. 2000. 16 Regulation of Macronutrient Partitioning between Maternal and Conceptus Tissues in the Pregnant Ruminant. RUMINANT PHYSIOLOGY: 275.

Bell, A. W. 1995. Use of ruminants to study regulation of nutrient partitioning during pregnancy and lactation. In: Symposium on the Animal Model for Human Research, Ottawa, Canada. p 41-62.

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proceedings of the Nutrition Society 59: 119-126.

Bell, A. W., C. L. Ferrell, and H. C. Freetly. 2005. Pregnancy and Fetal Metabolism. In: J. Dijkstra, J. M. Forbes and J. France (eds.) Quantitative aspects of ruminant digestion and metabolism No. 1. p 523-550. CAB International, Oxfordshire.

Bell, A. W., R. Slepetis, and R. A. Enrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy. Sci. 78: 1954-1961.

Bohnert, D. W. et al. 2013. Late gestation supplementation of beef cows differing in body condition score: Effects on cow and calf performance. J. Anim. Sci. 91: 5485-5491.

Crookenden, M. et al. 2015. Source of metabolizable energy affects gene transcription in metabolic pathways in adipose and liver tissue of nonlactating, pregnant dairy cows. J. Anim. Sci. 93: 685-698.

Detmann, E. et al. 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis–Menten kinetics. Livestock Science 126: 136-146.

Doelman, J. et al. 2012. Transcript profiling of the ruminant liver indicates a unique program of transcriptional regulation of ketogenic enzymes during food restriction. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 7: 303-310.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy. Sci. 84: E100-E112.

Duarte, M. S. et al. 2013. Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation. Livestock Science 153: 60-65.

Gionbelli, M. P. et al. 2015. Achieving Body Weight Adjustments for Feeding Status and Pregnant or Non-Pregnant Condition in Beef Cows. PloS one 10: e0112111.

Kalhan, S. C., and S. E. Marczewski. 2012. Methionine, homocysteine, one carbon metabolism and fetal growth. Reviews in Endocrine and Metabolic Disorders 13: 109-119.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. methods 25: 402-408.

McNeill, D. M., R. Slepetis, R. A. Ehrhardt, D. M. Smith, and A. W. Bell. 1997. Protein requirements of sheep in late pregnancy: partitioning of nitrogen between gravid uterus and maternal tissues. J. Anim. Sci. 75: 809-816.

Meijer, A. J. 2003. Amino acids as regulators and components of nonproteinogenic pathways. The Journal of nutrition 133: 2057S-2062S.

427 Mordhorst, B., C. Zimprich, L. Camacho, M. Bauer, and K. Vonnahme. 2017.
428 Supplementation of distiller's grains during late gestation in beef cows
429 consuming low-quality forage decreases uterine, but not mammary, blood flow.
430 Journal of animal physiology and animal nutrition 101.
431 Nicholson, M. J., and M. H. Butterworth. 1986. A guide to condition scoring of zebu
432 cattle. International Livestock Centre for Africa, Addis Ababa, Ethiopia.
433 Stalker, L. A., L. A. Ciminski, D. C. Adams, T. J. Klopfenstein, and R. T. Clark. 2007.
434 Effects of weaning date and prepartum protein supplementation on cow
435 performance and calf growth. Rangeland ecology & management 60: 578-587.
436 Trece, A. S. 2017. AVALIAÇÃO NUTRICIONAL E METABÓLICA EM VACAS DE
437 CORTE SUPLEMENTADAS NO PRÉ E/OU PÓS PARTO, Universidade
438 Federal de Viçosa.
439 Vonnahme, K. A., C. O. Lemley, J. S. Caton, and A. M. Meyer. 2015. Impacts of
440 maternal nutrition on vascularity of nutrient transferring tissues during gestation
441 and lactation. Nutrients 7: 3497-3523.
442 Winterholler, S. et al. 2012. Supplementation of dried distillers grains with solubles to
443 beef cows consuming low-quality forage during late gestation and early
444 lactation. J. Anim. Sci. 90: 2014-2025.
445 Wood, K. et al. 2013. Influence of pregnancy in mid-to-late gestation on circulating
446 metabolites, visceral organ mass, and abundance of proteins relating to energy
447 metabolism in mature beef cows. J. Anim. Sci. 91: 5775-5784.
448 Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino acids 37: 1-17.
449 Zhou, Z. et al. 2016. Circulating amino acids in blood plasma during the peripartal
450 period in dairy cows with different liver functionality index. J. Dairy. Sci. 99:
451 2257-2267.

452

453 **Table 1 Nutrient composition (percentage) and forage availability (ton/hectare) of feedstuffs fed to cows**
454 **receiving or not receiving a crude protein supplementation in late gestation**

Item	Crude protein Supplement	Forage
kg/ha		
Forage Mass	-	4750
pdDM	-	2710
%		
DM	87.40	41.08
CP	43.49	5.36
apNDF	19.25	72.80
apADF	6.83	34.88
iNDF	4.45	32.55
LIG	-	6.07

455 CP, crude protein; apNDF, neutral detergent fiber corrected to ash and protein; apADF,
456 acid detergent fiber corrected to ash and protein; iNDF, indigestible fiber detergent
457 neutral; LIG, lignine; pdDM, potentially digestible dry matter.

458 **Table 2 Sequences (5' to 3') and efficiencies of the primers used in quantitative real-time PCR**

Symbol	Forward and Reverse	Access Number	Tissue
ACACA	F AACATCCCCACGCTAAACAG R GAGTCATGCCGTAGTGGTTG	NM_174224	Liver
GHR1A	F TCCAGCCTCTGTTTCAGGAG R GCTGCCAGAGATCCATACCT	AY748827	Liver
IGF-1	F ATGTACTGCGCGCCTCTC R CCCTCTACTTGTGTTCTTCAAATG	NM_001077828	Liver
PC	F GAGGTGGTCCGCAAGATG R TCGTGCAGGGAAGTGATG	NM_177946	Liver
PCK1	F TGGATGAAATTTGACCAACAAG R GATTTGTCCTCACAGAGGTTCC	NM_174737	Liver
p70S6K	F TTGAACCAAAAATCCGATCC R AGCACCTCTTCCCCAGAAA	AY396564.1	Muscle
GSK3β	F GCCCAGAACCACCTCCTTT R TGCTGCCATCTTTGTCTCTG	NM_001101310.1	Muscle
eIF4E	F AAACCACCCCTACTCCGAAT R TGCCCATCTGTTCTGTAAAGG	NM_174310	Muscle
MuRF1	F GGGACAGATGAGGAAGAGGA R CCTCATCATCGCCTTACTGG	NM_001046155.1	Muscle
Atrogin 1	F CCTTGAAGACCAGCAAAACA R AGACTTGCCGACTCTTTGGA	NM_001046155.1	Muscle
18 S	F CCTGCGGCTTAATTTGACTC	NM_001033614	Muscle and Liver

	R AACTAAGAACGGCCATGCAC		
GAPDH	F CGACTTCAACAGCGACACTC R TTGTCGTACACAAGGAAATGAGC	NM_001034034.1	Muscle

PCK1, phosphoenolpyruvate carboxykinase 1; GHR1A, growth hormone receptor 1[a]; IGF1, insulin-like growth factor 1; ACACA, acetyl-CoA carboxylase alpha; PC, pyruvate carboxylase; GSK3B, glycogen synthase kinase 3β; eIf4E, eukaryotic translation initiation factor 4E; MuRF1, muscle ring finger 1; p7056k, ribosomal protein S6 kinase; 18S (18S ribosomal); GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 3 Measures of cows receiving or not receiving a crude protein supplementation in late pregnancy and her calves.

Measure	Treatment		SEM	P-Value
	Control	Supplement		
Cow				
ajBWP	503.6	517.3	5.98	0.160
ADG	0.090	0.143	0.04	0.450
conADG	0.494	0.438	0.06	0.548
BCC	5.49	5.59	0.10	0.542
Calf				
BW	36.9	36.2	1.45	0.649

ajBWP, body weight adjusted to parturition; ADG, average daily gain; conADG, concept average daily gain; BCC, body condition score; BW, birth weight.

Table 4 Groups of total, Essential, Nonessential, Glucogenic, Ketogenic, Glucogenic and Ketogenic, and individual essentials and nonessentials circulating plasma amino acids of cows receiving or not receiving a crude protein supplementation in late pregnancy.

	Treatment		SEM	P-Value
Amino acid, nmol/mL	Control	Supplement		
Total AA	1883.82	1661.01	74.09	0.057
Essential AA	1027.72	917.83	49.45	0.146
Nonessential AA	538.92	472.38	24.68	0.084
Glucogenic AA	1364.46	1193.85	50.33	0.035
Ketogenic AA	166.34	152.54	8.05	0.254
Glucogenic and Ketogenic AA	353.02	314.61	18.87	0.180
Branched-Chain AA	669.24	608.33	34.99	0.250
Essential				
Arginine	89.58	81.48	13.60	0.223
Phenylalanine	69.00	63.48	3.32	0.268
Histidine	114.84	88.81	6.38	0.015
Isoleucine	162.1	140.03	9.21	0.120
Methionine	37.96	29.8	1.74	0.007
Leucine	166.34	152.54	8.05	0.254
Tryptophan	47.10	45.92	3.01	0.789

Valine	340.80	315.75	18.41	0.360
Nonessential				
Aspartate	4.90	4.91	0.49	0.988
Glutamate	44.48	41.08	3.12	0.460
Asparagine	42.66	36.03	2.16	0.052
Serine	76.36	63.91	4.31	0.066
Glutamine	295.70	261.28	13.60	0.102
Tyrosine	74.82	65.17	5.35	0.231
Alanine	317.18	270.80	13.76	0.036

Table 5 Relative gene expression in liver of cows receiving or not receiving a crude protein supplementation in late pregnancy.

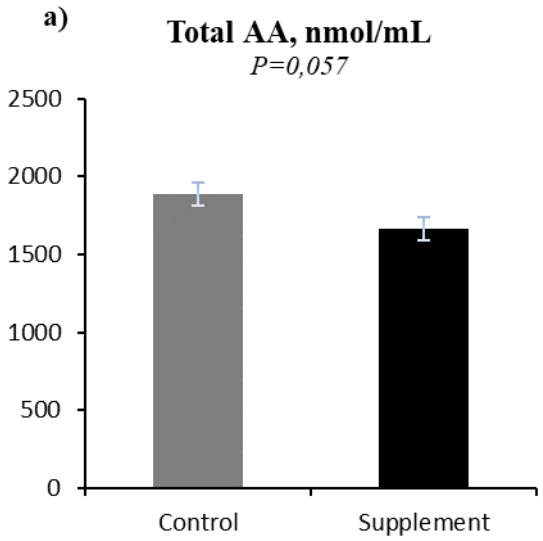
Gene	Relative expression		SEM	*P*-value
	Control	Supplement		
PCK1	3.27	2.92	0.448	0.598
GHR1A	4.54	4.53	0.408	0.993
IGF1	9.22	9.26	0.938	0.975
ACACA	6.00	6.22	0.847	0.872
PC	2.82	2.55	0.455	0.690

PCK1, phosphoenolpyruvate carboxykinase 1; GHR1A, growth hormone receptor 1[a]; IGF1, insulin-like growth factor 1; ACACA, acetyl-CoA carboxylase alpha; PC, pyruvate carboxylase.

Table 6 Relative gene expression in skeletal muscle of cows receiving or not receiving a crude protein supplementation in late pregnancy.

Gene	Relative expression		SEM	*P*-value
	Control	Supplement		
GSK3B	3.48	4.50	0.365	0.096
eIF4E	2.78	3.34	0.315	0.253
MuRF1	2.83	3.69	0.369	0.153
P7056k	3.09	4.11	0.314	0.060
Antrogin	2.48	2.79	0.253	0.424

GSK3B, glycogen synthase kinase 3β; eIf4E, eukaryotic translation initiation factor 4E; MuRF1, muscle ring finger 1; p7056k, ribosomal protein S6 kinase.



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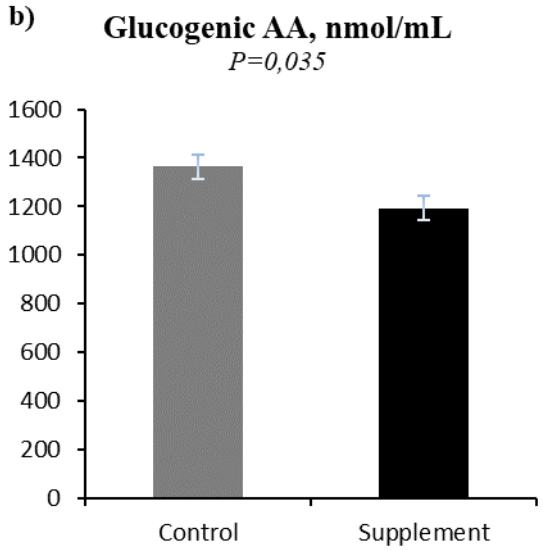

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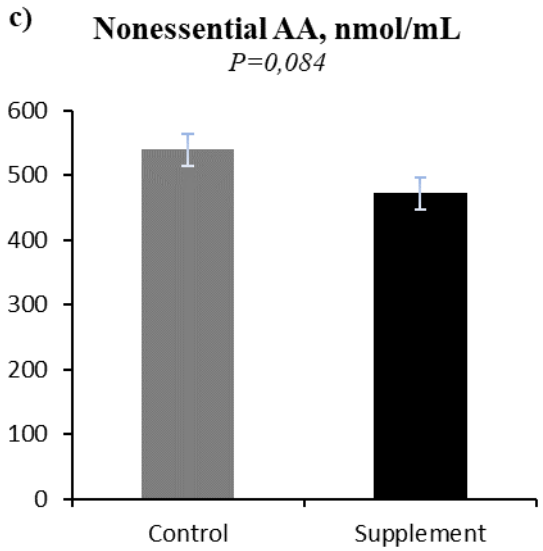

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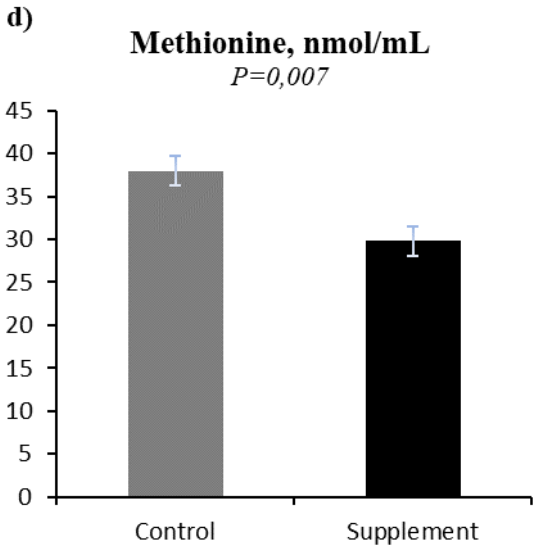

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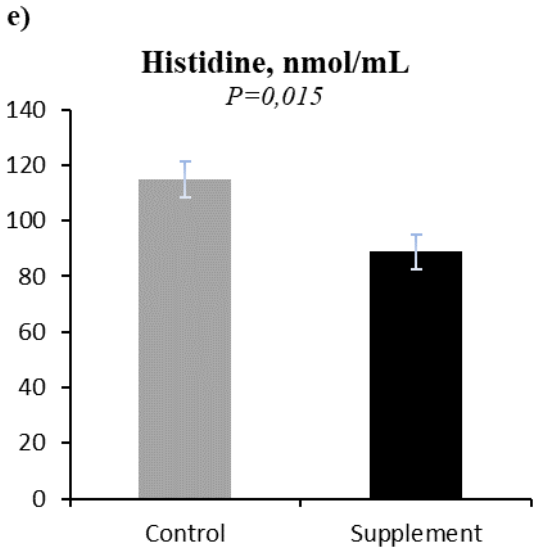

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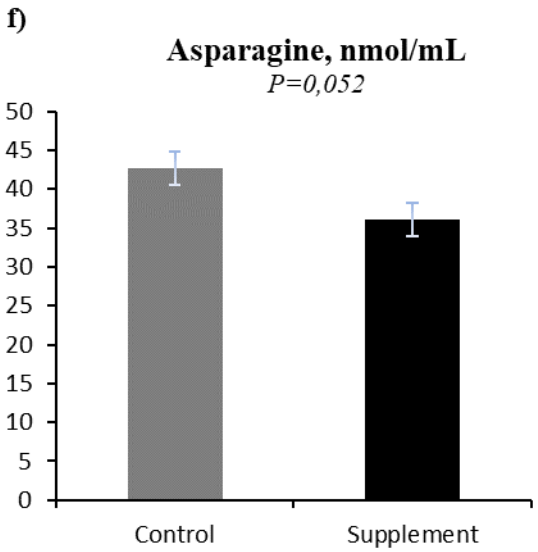

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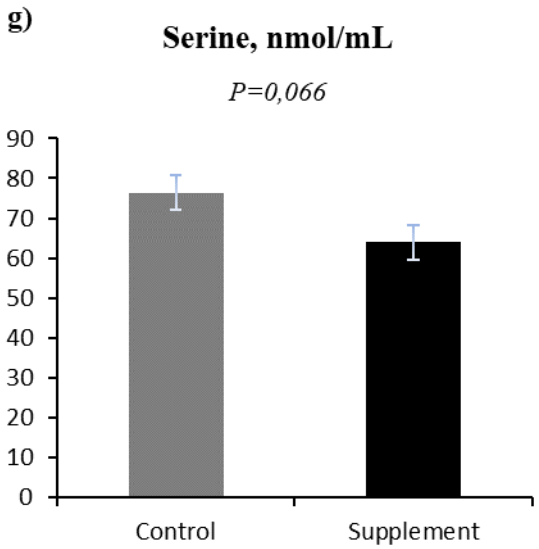

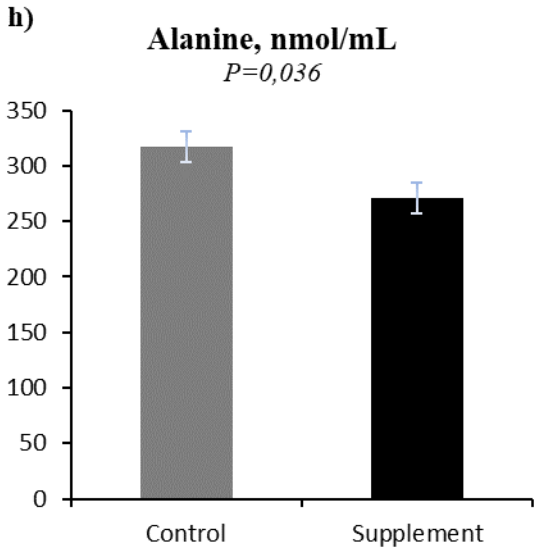

Figure 1 Total (a), glucogenic (a) and nonessential (c), and individual circulating plasma amino acids as methionine (d), histidine (e), asparagines (f), serine (g) and Alanine (h) of cows receiving or not receiving a crude protein supplementation in late gestation.

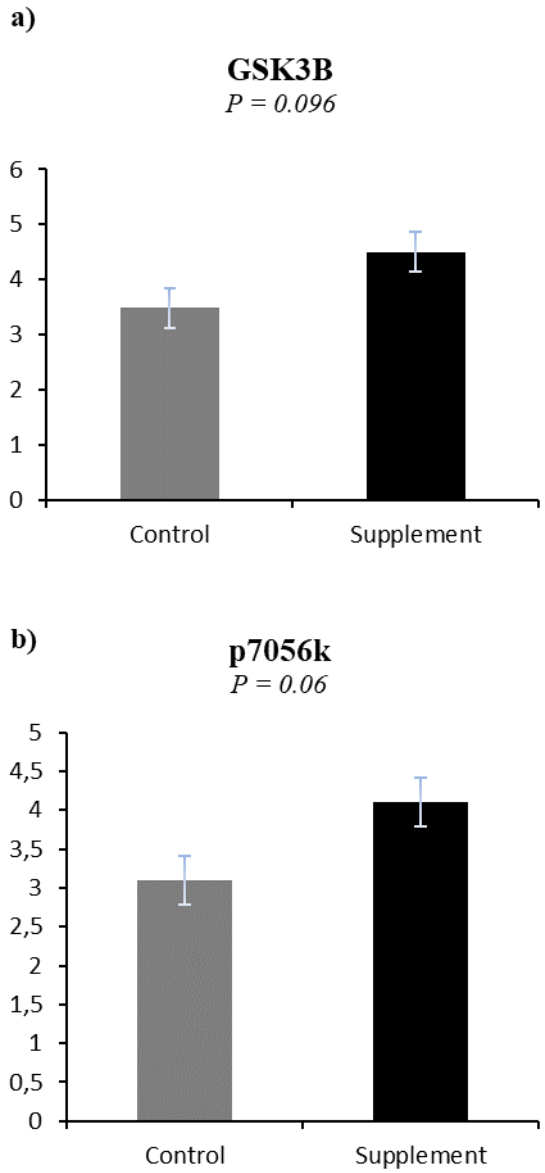

Figure 2 Relative gene expression of glycogen synthase kinase 3β (a) and ribosomal protein S6 kinase (b) in skeletal muscle of cows receiving or not receiving a crude protein supplementation in late gestation.