MATEUS PIES GIONBELLI

NUTRIENT REQUIREMENTS AND QUANTITATIVE ASPECTS OF GROWTH, DEVELOPMENT AND DIGESTION OF PREGNANT AND NON-PREGNANT NELLORE COWS

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

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In memory of my brother ***Cristian Pies Gionbelli***,
whose example is my inspiration. Cristian taught me
the meaning of love, pain, peace and hopefulness.

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For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own!

BIOGRAPHY

Mateus Pies Gionbelli, son of Valdir Gionbelli and Cecília Pies Gionbelli, was born in Campo Erê/SC-Brazil on July 12, 1986.

He started the undergrad in Animal Science at *Universidade do Estado de Santa Catarina* in 2004 and became a Bachelor of Science in Animal Science in 2008. At the same year he started the M.S. program with major on ruminant nutrition and beef cattle production.

In February of 2010 he became a M.S. in Animal Science. At the same year he started his D.S. program in Animal Science with major on ruminant nutrition and beef cattle production.

From March of 2012 to September of 2012 he was a visiting scholar at Ruminant Nutrition Unit of the United States Meat Animal Research Center, Clay Center/NE, USA where part of his research was developed.

On September 5th of 2013 Mr. Gionbelli submitted his dissertation to the thesis committee to obtain the *Doctor Scientiae* degree in Animal Science.

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ABSTRACT

GIONBELLI, Mateus Pies, D.Sc., Universidade Federal de Viçosa, September of 2013. **Nutrient requirements and quantitative aspects of growth, development and digestion of pregnant and non-pregnant Nellore cows.** Adviser: Sebastião de Campos Valadares Filho. Co-Advisers: Mário Luiz Chizzotti and Edenio Detmann.

Pregnancy is the most complex physiological stage of a cow production cycle. Several challenges occur in the normal physiology status of the cow to support the formation of a new individual of the species. Data about the nutrient requirements and quantitative aspects of growth, development and digestion of pregnant *Bos indicus* cows are limited. Aiming to fill at least part of the existing knowledge gap, we conducted a comparative slaughter experiment with pregnant and non-pregnant Nellore cows. The results are presented in four chapters. The objective of the work described in the first chapter was to evaluate the growth and development of maternal and fetal organs and tissues during pregnancy in zebu cows and the effect of feeding level on tissue development. Forty-nine multiparous Nellore cows (32 pregnant and 17 non-pregnant) with average initial body weight of 451 ± 10 kg were used. Cows were segregated into 2 groups according to level of feeding; either HIGH (*ad libitum*) or LOW (restricted feeding 1.2 times maintenance according to the NRC). The 32 pregnant cows were separated into 4 groups of 8 cows each and harvested at 136, 189, 239 and 269 d of pregnancy for tissue collection and analysis. HIGH-fed cows had greater BW gain ($P < 0.01$), final BW ($P < 0.01$) and body condition score ($P < 0.01$). In early gestation, LOW-fed and HIGH-fed cows had similar accretion of fat in the empty body free of pregnancy components (EBW_{np}), however, with the increase in gestational age, the daily rates of fat gain were greater in HIGH-fed cows than those in LOW-fed cows. The BW gain in pregnant LOW-fed cows was mostly fat while HIGH-fed cows accumulated also other types of tissues. LOW-fed cows had lower proportion of organs and viscera (139 vs 159 g/kg of

EBW_{np}, $P < 0.01$). The interaction between feeding level and time of gestation (P = 0.03) showed that the gravid uterus was greater in HIGH-fed cows only in late gestation, although there were no differences in chemical composition and energy content of gravid uterus due to feeding level ($P > 0.10$). HIGH-fed cows increased the weight of fresh udder after 239 d of gestation ($P < 0.10$) while LOW-fed cows did not present significant udder increase due to pregnancy ($P > 0.10$). LOW-fed cows had proportionally (g/kg of EBW_{np}) lower liver, visceral fat and mesentery ($P < 0.01$) and greater heart ($P < 0.10$) than HIGH-fed cows. The proportional weights of rumen-reticulum, omasum and total stomach were affected ($P \leq 0.05$) by pregnancy and were lower at 269 d than in early gestation. HIGH-fed cows had greater fetuses at 269 d of gestation than LOW-fed cows. Fetuses from HIGH-fed cows had greater carcasses (P = 0.03) but did not differ from fetuses from LOW-fed cows in organs and viscera (P = 0.27). Placenta weight was greater in HIGH-fed cows ($P < 0.01$) and the relationship between placenta and fetus weight was different between feeding levels ($P < 0.01$). The weights of the gravid uterus and fetus, as well as their contents of energy and N, were lower in this study compared to previous studies with *Bos taurus* cattle. In the second chapter, data from the same 49 cows used in the first chapter were used to develop a set of equations and relations for BW adjustments in pregnant or not pregnant beef cows, improving the options and the reach of the research in the area. Cows were weighed every 28 d (0700 h, before feeding) to obtain the BW, and reweighed at the same time the following day after 16 h of fasting to obtain the shrunk body weight (SBW) values. Pregnant cows were separated into four groups of eight cows each and harvested at 136, 189, 239 and 269 d of pregnancy to obtain the empty body weight (EBW) and the weight of components related to pregnancy. To establish the relationships between the BW, SBW and EBW of pregnant and non-pregnant cows as a function of time of gestation, a set of general equations was tested, based on theoretical suppositions. The pregnant compound (PREG) was defined as the weight that is genuinely related to pregnancy, that includes the gravid uterus minus the non-pregnant uterus plus the accretion in udder related to pregnancy. The PREG was deducted from the SBW or EBW of a pregnant cow to estimate the non-pregnant SBW and EBW (SBW_{np} and EBW_{np}) and to calculate the body gain of only maternal tissues (rSBG and rEBG). The overall equation to predict SBW from BW was $0.8084 \times BW^{1.0303}$. The equation to predict gravid uterus weight in function of days of pregnancy (DOP) was $0.2106 \times e^{((0.03119 - 0.00004117 \times DOP) \times DOP)}$. There was no accretion of weight in udder up to 238 d of pregnancy and the accretion after 238 d was exponentially correlated with DOP. We conclude that the weight related to the pregnancy can be estimated in a live cow allowing estimating the non-pregnant EBW and SBW of a pregnant cow. This allows estimate and compare the weight gain of tissues not related to the pregnancy and the comparison of weight gain of a pregnant and non-pregnant cow. The objective of the third chapter was to evaluate the effects of pregnancy and feeding level on intake, digestibility and efficiency of microbial N production in Nellore cows. Forty-four multiparous Nellore cows (32 pregnant and 12 non-pregnant) with average initial body weight of 451 ± 10 kg were fed either HIGH (*ad libitum*) or LOW (restricted feeding 1.2 times maintenance according to the NRC) feeding level. The diet consisted of corn silage (85%), ground corn, soybean meal, urea and mineral mixture. The intake was controlled daily and the DM intake (DMI) was evaluated weekly. In vivo apparent total digestibility was estimated using indigestible NDF as an internal marker and microbial N synthesis was estimated from the technique of the purine derivatives in urine. The voluntary feed intake reduced as the pregnancy advances in Nellore cows and can be calculated as DMI (kg/d) = (16 – 0.0093 × days in pregnancy) / 1000 × SBW_p or DMI (kg/d) = (16.4 – 0.0093 × days in pregnancy) / 1000 × SBW_{np}. The average DMI of LOW-fed cows corresponded to 102, 98 and 67% of the amount of energy necessary to attend the maintenance and pregnancy energy requirements suggested by NRC for a cow at 0, 135 and 270 d of pregnancy, respectively. LOW-fed cows had 0.26 kg/d of average shrunk body gain indicating that the nutrient energy requirements of Zebu cows are likely lower than those suggested by NRC. The interaction between the feeding level and days of pregnancy was significant ($P<0.05$) for the digestibility of DM, OM, N, EE, NDF_{ap} and GE, and the values of TDN. In all these cases there was a reduction in digestibility with increasing gestation age in HIGH-fed cows, while the digestibility OM, N, EE, NDF_{ap} and GE increased as function of days of pregnancy in LOW-fed. The reduction in the digestibility of neutral detergent fiber occurs faster than in dry matter digestibility. These data suggests that the reduction of the digestibility as pregnancy increases is caused by an increase in the rate of passage as compensation factor for the ruminal volume reduction. There were no direct effects of pregnancy on microbial N production in Nellore cows. In the fourth chapter 49 adult Nellore cows (32 pregnant and 17 non-pregnant) with average initial body weight of 451 ± 10 kg were used in a comparative slaughter study aiming to describe equations and relationships for prediction of net, metabolizable and dietary energy and protein requirements for adult, pregnant and non-pregnant, *Bos indicus* cows. Feeding control was measured individually and cattle were fed either HIGH (*ad libitum*) or LOW (restricted feeding 1.2 times maintenance according to the NRC). The 32 pregnant cows were separated at random into 4 groups of 8 cows each (4 cows per each feeding level) and harvested at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. The non-pregnant cows were harvested at different times of the experiment (85 to 216 days of feeding control) in order to keep them in experiment for a similar amount of time as the pregnant cows.

The digestible energy and N and losses of energy as methane and urine were directly measured to establish the relations between GE, DE and ME. Energy and N content were analyzed in empty body and pregnant compounds and a set of relationships and equations based in the factorial method from ARC was used to estimate the nutrient requirements of energy and N. The net energy and protein requirements for pregnancy (NE_p and NP_p) estimated in this study were about ¾ of those estimated by NRC. When estimated by a logistic model, the daily requirements for pregnancy showed an exponential increase up to approx. 250 days of gestation and then decreased. However, when an allometric model was used to estimate the daily requirements for pregnancy, the maximum daily requirements were at birth. There were no differences in the dynamics of energy and protein (P=0.388 and 0.137, respectively) in the cow's empty body weight pregnant free (EBW_{np}) suggesting that the pregnancy does not affect the requirements for accretion of body reserves in cows. The partial efficiencies for use of metabolizable energy for maintenance, weight gain and pregnancy (k_m, k_g and k_c) were respectively 70, 53 and 12%. The partial efficiencies for use of metabolizable protein for maintenance, weight gain and pregnancy (z_m, z_g and z_c) were respectively 22, 22 and 5%, indicating that in mature cows the major portion of metabolizable protein intake is not used for tissue accretion. The efficiency of transformation of DE in ME was 0.80.

RESUMO

GIONBELLI, Mateus Pies, D.Sc., Universidade Federal de Viçosa, setembro de 2013. **Requerimentos nutricionais e aspectos quantitativos do crescimento, desenvolvimento e digestão de vacas Nelore gestantes e não gestantes.** Orientador: Sebastião de Campos Valadares Filho. Coorientadores: Mário Luiz Chizzotti e Edenio Detmann

A gestação é o mais complexo estágio fisiológico de uma vaca durante um ciclo produtivo. Vários desafios ocorrem no status fisiológico normal da vaca para suportar a formação de um novo indivíduo da espécie. Dados sobre exigências nutricionais e aspectos quantitativos do crescimento, desenvolvimento e digestão de vacas *Bos indicus* são limitados. Objetivando preencher pelo menos uma parte da lacuna existente, foi conduzido um experimento de abate comparativo com vacas gestantes e não gestantes. Os resultados são aqui apresentados em quatro capítulos. No primeiro capítulo, quarenta e nove vacas Nelore (32 gestantes e 17 não gestantes) com peso médio inicial de 451 ± 10 kg foram usadas num experimento para avaliar o crescimento e desenvolvimento de tecidos maternos e fetais durante a gestação em vacas zebuínas. As vacas foram divididas em dois grupos de acordo com o nível alimentar, que foi ALTO (ad libitum) ou BAIXO (alimentação restrita a 1,2 vezes a mantença, segundo o NRC). As 32 vacas gestantes foram separadas em 4 grupos com 8 vacas em cada e abatidas aos 136, 189, 239 e 269 dias de gestação para coletas de tecidos e análises. Vacas do alto nível alimentar tiveram maior ganho de peso ($P<0,01$), peso final ($P<0,01$) e escore de condição corporal ($P<0,01$). No início da gestação, as vacas dos dois grupos alimentares tiveram similar acréscimo de gordura no peso de corpo vazio isento de componentes relativos à gestação ($PCVZ_{np}$). Entretanto, com o aumento da gestação, as taxas diárias de ganho de gordura foram maiores nas vacas de alto nível alimentar do que nas do nível baixo. O ganho de peso obtido por vacas do nível alimentar baixo foi praticamente apenas gordura, enquanto que vacas do alto nível alimentar acumularam também outros tipos de tecidos. Vacas do baixo nível alimentar tiveram menor proporção de órgãos e

víscera (139 vs 159 g/kg de $PCVZ_{np}$, P<0.01). A interação entre o nível alimentar e o tempo de gestação (P=0,03) mostrou que o útero grávido foi maior nas vacas do nível alimentar alto no final da gestação, embora não fossem observadas diferenças significativas na composição química e conteúdo de energia do útero grávido em função do nível alimentar (P>0,10). Vacas do nível alimentar alto aumentaram o peso do úbere após 239 dias de gestação (P<0,10) enquanto que vacas do nível alimentar baixo não apresentaram aumento significativo. Vacas do menor nível alimentar tiveram proporcionalmente menores pesos de fígado, gordura visceral e mesentério (P<0,01) e maior coração (P<0,10) do que vacas do nível alimentar alto. Os pesos proporcionais do rúmen-retículo e dos estômagos foram afetados (P<0,05) pela gestação e foram menores aos 269 dias de gestação do que no início da gestação. Vacas do nível alimentar alto tiveram fetos mais pesados aos 269 dias de gestação do que vacas do nível alimentar baixo. Os fetos das vacas do maior nível alimentar tiveram maiores carcaças (P=0,03), mas não diferiram dos fetos das vacas do menor nível alimentar no peso de órgãos e vísceras (P=0,27). O peso da placenta foi maior em vacas do maior nível alimentar (P<0,01) e a relação entre peso da placenta e do feto foi diferente entre os níveis alimentares (P<0,01). Os pesos do útero grávido e fetos, como também seus conteúdos de energia e nitrogênio, foram menores neste estudo do que em estudos prévios realizados com gado *Bos taurus*. No segundo capítulo, dados das mesmas vacas utilizadas no primeiro capítulo foram utilizados para desenvolver um grupo de equações e relações para ajuste de peso corporal em vacas gestantes e não gestantes, ampliando as opções para a pesquisa na área de gado de corte. A cada 28 dias as vacas foram pesadas pela manhã (7h, imediatamente antes da alimentação) para obter o peso vivo (PV), e repesadas ao mesmo horário no dia seguinte após 16 horas de jejum de sólidos para obter-se o peso vivo em jejum (PVJ). As vacas gestantes foram separadas em 4 grupos de 8 vacas cada e abatidas aos 136, 189, 239 e 269 dias de gestação para obter o peso de corpo vazio (PCVZ) e o peso dos componentes relacionados à gestação. Para estabelecer-se as relações entre PC, PCJ e PCVZ de vacas gestantes e não gestantes em função do tempo de gestação, um grupo de equações gerais foi avaliado, baseado em suposições teóricas que foram apresentadas com suas explanações. Sugeriu-se o conceito de componente gestação (PREG), que representa o útero grávido menos o peso do útero numa condição não gestante mais o acréscimo de úbere que ocorreu devido à gestação. A partir disso, o PREG foi subtraído dos valores de PVJ e PCVZ das vacas gestantes para estimar-se os PVJ e PCVZ não gestantes (PVJ_{np} e $PCVZ_{np}$) e para calcular-se o ganho de peso relativo apenas aos tecidos maternos (rGPVJ e rGPCVZ). A equação gerada para predizer PVJ a partir do PV foi $0,8084 \times BW^{1,0303}$. A equação gerada para predizer o peso do útero grávido em função dos dias de gestação (DG) foi $0.2106 \times e^{((0.03119 - 0.00004117 \times DG) \times DG)}$. Não foi observado acréscimo no peso do úbere até 238 dias de gestação e após isso o acréscimo foi exponencialmente correlacionado com DG. A aplicação das equações e relações geradas pode ser útil como uma ferramenta prática para separar a porção do ganho diário e peso vivo é referente à tecidos maternos e a porção relativa à gestação. O objetivo do estudo apresentado no terceiro capítulo foi avaliar os efeitos da gestação e do nível alimentar sobre o consumo, a digestibilidade e a eficiência de produção microbiana em vacas Nelore. Quarenta e quatro vacas Nelore adultas, multíparas (32 gestantes e 12 não gestantes) com peso médio inicial de 451 ± 10 kg foram alimentadas a um nível alto (ad libitum) ou baixo (alimentação restrita a 1,2 vezes a mantença, de acordo com o NRC) da mesma dieta. A dieta consistiu de silagem de milho (85%), milho grão moído, farelo de soja, uréia e mistura mineral. O consumo foi controlado diariamente e o consumo diário de matéria seca (CMS) foi avaliado semanalmente. A digestibilidade aparente in vivo foi estimada usando-se FDNi como marcador interno da digesta. A produção de N microbiano foi avaliada pela técnica dos derivados de purina na urina. O consumo voluntário reduziu com o aumento da gestação e pode ser descrito como CMS (kg/d) = (16 – 0,0093 × dias de gestação) / 1000 × PCVZ ou CMS (kg/d) = (16,4 – 0,0093 × dias de gestação) / 1000 × $PCVZ_{np}$. O CMS médio das vacas do baixo nível alimentar correspondeu a 102, 98 e 67% da quantidade de consumo necessário para atender as exigências de energia sugeridos pelo NRC para uma vaca aos 0, 135 e 270 dias de gestação, respectivamente. Vacas do baixo nível alimentar tiveram ganho médio diário de 0,26 kg, indicando que as exigências de energia estimadas pelo NRC são superestimadas para vacas zebuínas. A interação entre o nível alimentar e o tempo de gestação foi significativa (P<0,05) par as digestibilidades da matéria seca, matéria orgânica, nitrogênio, extrato etéreo, fibra em detergente neutro e energia bruta, e também para os valores de NDT. Em todos os casos, houve redução da digestibilidade em função do aumento da gestação nas vacas alimentadas ad libitum, enquanto que nas vacas que sofreram restrição alimentar as digestibilidades de matéria orgânica, nitrogênio, extrato etéreo e fibra em detergente neutro aumentaram em função do tempo de gestação. A redução da digestibilidade da fibra em detergente neutro em função do tempo de gestação ocorreu mais rapidamente do que da digestibilidade da matéria seca. Os dados desse estudo sugerem que a redução na digestibilidade ocorrida em função do tempo de gestação nas vacas alimentadas ad libitum tenha sido causada pelo aumento da taxa de passagem, como fator compensatório para a redução do volume ruminal. Não foram observados efeitos da gestação sobre a produção de N microbiano em vacas Nelore. No quarto capítulo 49 vacas Nelore adultas (32 gestantes e 17 não gestantes) com peso médio inicial de 451 ± 10 kg foram usadas num experimento de abate comparativo objetivando descrever equações e relações para predizer as exigências líquidas e metabolizáveis de energia e proteína para vacas zebuínas adultas, gestantes e não gestantes. O consumo de alimento foi mensurado individualmente, sendo as vacas alimentadas com alto (ad libitum) ou baixo nível alimentar (1,2 vezes a mantença, segundo o NRC). As 32 vacas gestantes foram separadas em 4 grupos com 8

vacas cada (4 de cada nível alimentar) e abatidas aos 136 ± 1, 189 ± 1, 239 ± 1 e 269 ± 1 dias de gestação. As vacas não gestantes foram abatidas em diferentes tempos de experimento (85 a 216 dias de controle experimental) objetivando mantê-las em experimento por tempo semelhante às vacas gestantes. A energia e N digestível, bem como as perdas de energia na forma de metano e urina foram diretamente mensuradas para estabelecer as relações entre energia bruta, energia digestível e energia metabolizável. Os conteúdos de energia e N foram diretamente analisados no corpo vazio e no componente gestação das vacas. Um conjunto de equações e relações baseadas no método fatorial foi utilizado para estimar as exigências nutricionais de energia e proteína. Os requerimentos líquidos de energia e proteína para gestação (EL_p e PL_p) estimados nesse estudo foram em média ¾ dos do NRC. Quando estimados por um modelo logístico, os requerimentos para gestação aumentaram até aproximadamente 250 dias de gestação, e então reduziram. Entretanto, quando estimados por modelos alométricos simples, os requerimentos para gestação atingiram o máximo somente próximo ao parto. Não foram observadas diferenças na dinâmica de energia e proteína (P=0,388 e 0,137, respectivamente) no corpo vazio da vaca livre do componente gestação, sugerindo que a gestação não afeta as exigências nutricionais para acumulo de reservas corporais em vacas adultas. As eficiências parciais de uso da energia metabolizável para mantença, ganho de peso e gestação (k_m, k_g e k_c) foram, respectivamente, 70, 53 e 12%. As eficiências parciais de uso da proteína metabolizável para mantença, ganho de peso e gestação (z_m, z_g e z_c) foram respectivamente 22, 22 e 5%, indicando que em vacas adultas grande parte da proteína metabolizável não é recuperada na forma de tecidos. A eficiência de transformação da energia digestível em energia metabolizável foi 0,80.

INTRODUÇÃO GERAL

A única categoria na qual ainda não se dispõe de conhecimentos mínimos sobre requerimentos nutricionais de zebuínos é a de vacas em gestação. Embora seja clara a importância do conhecimento dos requerimentos nutricionais dessa categoria, não foram observados na literatura mundial trabalhos envolvendo a quantificação das exigências nutricionais para mantença e gestação de vacas zebuínas.

O Brasil possui por volta de 67 milhões de matrizes de corte (Vasconcelos e Meneghetti, 2006), que fazem parte de um rebanho bovino de cerca de 200 milhões de animais, das quais estima-se que 80% possuam algum grau de sangue da raça Nelore (ANUALPEC, 2011). Esse mesmo rebanho ocupa cerca de 172 milhões de hectares de pastagens (IBGE, 2011), dos quais por volta de metade é ocupada pelos rebanhos de cria.

Segundo Ritchie (1995), 71% do total de energia gasto na produção de gado de corte é usado para mantença e 70% dessa energia de mantença é consumida pelo rebanho de cria. Portanto, 50% da energia exigida para a produção de gado de corte são demandados para mantença das vacas. Se a isto acrescentarmos as demandas para reprodução e para lactação, ter-se-á que cerca de 60 a 70% de toda a energia do sistema são gastos na fase de cria.

Dessa forma, o conhecimento das exigências de energia, proteína e minerais, bem como as eficiências de utilização da energia e nutrientes de vacas de corte gestantes para as condições brasileiras representaria um grande avanço para a pesquisa na área da ciência animal no Brasil. O conhecimento destas informações permitiria o desenvolvimento de tecnologias de produção mais eficientes e daria base científica para o direcionamento das decisões a serem tomadas no meio produtivo relacionado a essa categoria animal que, conforme comentado, é responsável pelo gasto de mais da metade da energia consumida para a produção de carne no Brasil.

Trabalhos nos quais se realizou abate comparativo para quantificar as exigências nutricionais de vacas em gestação são bastante escassos na literatura mundial, de forma que os principais sistemas de exigências nutricionais (ARC, 1980; AFRC, 1993; NRC, 2000; INRA, 2007; CSIRO, 2007) baseiam suas recomendações em alguns poucos trabalhos realizados ou em estimativas indiretas e adaptações de valores obtidos com experimentos envolvendo ovinos. O ARC (1980) baseou suas recomendações emtrabalho envolvendo vacas Ayrshire e Jersey, realizado no ano de 1975, sendo que o AFRC (1993) não adotou atualizações significativas sobre a forma de calcular os requerimentos nutricionais para gestação. O NRC (2000) baseou suas recomendações nos trabalhos de Ferrell et al. (1976a, 1976b e 1976c), realizados com animais Hereford, sendo um dos poucos experimentos conhecidos no qual foi realizado abate comparativo com fêmeas em gestação. Adicionalmente, o NRC (2000) apresentou algumas sugestões de ajustes baseados no trabalho de Prior e Laster (1979), realizado com animais da raça Pardo Suíça. O sistema francês (INRA, 2007) possui estimativas de exigências nutricionais para gestação definidas desde sua edição de 1978, e baseia suas recomendações no trabalho de Ferrell et al. (1976a) e em trabalho sobre composição química de fetos bovinos realizado na França (Cano, 1995). As recomendações de requerimentos nutricionais para gestação apresentadas pelo sistema australiano (CSIRO, 2007) são baseadas nas indicações feitas pelo ARC (1980) e em ajustes e adaptações obtidos a partir de trabalhos realizados com ovinos, que existem em maior número na literatura.

O conceito de que fêmeas gestantes particionam os nutrientes disponíveis de forma a favorecer a sua prole foi inicialmente formulado por Hammond (1947), que sugeriu que os diferentes tecidos competem por nutrientes circulantes com base nas suas respectivas taxas metabólicas. Esta idéia foi reforçada pela descoberta de altas taxas metabólicas do útero grávido em relação ao corpo da matriz (Meschia et al., 1980). Entretanto, pesquisas mais recentes têm se concentrado na regulação endócrina dos tecidos ao invés da competição como um mecanismo explicativo de forma geral. Esta forma de pensamento advém do conceito de "homeorrese", elaborado por Bauman e Currie (1980). Este conceito sugere que há uma influência simultânea de múltiplos tecidos implicando mediação extracelular para que o metabolismo atenda às demandas de forma mais coerente em níveis que otimizem a oportunidade do feto crescer e sobreviver no pós-natal, e minimizando a excessiva depleção das reservas maternais de energia e proteína.

O crescimento do útero grávido ou concepto (feto, placenta, líquidos fetais, membranas fetais e tecidos de suporte do útero) no terço final da gestação exige suprimento materno de glicose e aminoácidos em grandes quantidades. Estudos do metabolismo do concepto desenvolvidos *in vivo* têm se concentrado principalmente no metabolismo fetal em termos de trocas umbilicais de oxigênio, nutrientes e metabólitos (Bell, 1993, 1995; Battaglia e Meschia, 1998; Bell et al., 2005). Cabe ressaltar também que a maioria desses estudos é realizado em ovelhas, e uma menor parte, em vacas.

Alguns modelos de padrões metabólicos para gestação foram desenvolvidos para ovelhas (Bell, 1993), vacas (Bell, 1995) e ambas espécies (Bell et al., 2005). Esses modelos prevêem que em ambas as espécies, 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 seria 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), restringindo o acesso fetal a substratos derivados da mobilização de gordura materna e, assim, restringindo a capacidade do feto de crescer às custas de reservas maternas de energia. Quase todo o nitrogênio adquirido pelo feto está na forma de aminoácidos, embora uma pequena absorção umbilical de amônia seja observada a partir da fase intermediária da gestação (Holzman et al., 1977; Bell et al., 1989).

Durante o terço final da gestação, os ruminantes, em geral, tendem a aumentar a ingestão voluntária de dietas de qualidade média a alta (Forbes, 1986). Isto direciona ao fígado maiores quantidades de substratos gliconeogênicos de origem alimentar (principalmente propionato e aminoácidos absorvidos). No entanto, 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 e Ferrell, 1998). Parte desse aumento na gliconeogênese é suportado pelo aumento da captação hepática de lactato (Freetly e Ferrell, 1998), aparentemente derivado do metabolismo e aumento da glicose útero-placentária materna em tecidos periféricos (Bell e 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 e Ferrell, 2000).

Em ruminantes, é sugerido que as fêmeas podem aumentar a ingestão voluntária de alimentos na metade da gestação, porém, esse aumento é muito menos pronunciado do que em porcas e muitas vezes não é notado (Ingvartsen e Andersen, 2000). Forbes (1986) relatou que vacas e ovelhas tendem a aumentar, por ficarem mais seletivas, o consumo voluntário de alimentos de maior qualidade nutricional quando o final da gestação se aproxima. Por outro lado, porém, há redução proeminente do consumo nas semanas finais da gestação de bovinos. Ingvartsen et al. (1992) sumariaram dados de 20 grupos de vacas de nove publicações, nos quais observam-se variações no consumo nas últimas semanas que vão desde aumento de 0,2%/semana até redução de 9,4%/semana. Os mesmos autores também verificaram que novilhas reduziram o consumo voluntário nas últimas 14 semanas de gestação em 1,53%/semana, com aumento dessa taxa nas duas últimas semanas, e cerca de 30% de redução nos cinco dias que antecedem o parto. As variações observadas no consumo durante a gestação também podem ser diferentes para multíparas e primíparas (Ingvartsen e Andersen, 2000).

A regulação da ingestão de alimentos por vacas em gestação pode apresentar fatores físicos e fisiológicos que não são contemplados em modelos tradicionais de regulação da ingestão de alimentos em ruminantes (Forbes, 1980; Fisher et al., 1987). Essas particularidades, como a influência do peso do bezerro na redução da capacidade ruminal, a regulação hormonal da gestação, ou ainda o mecanismo homeorrético de utilização dos nutrientes, são difíceis de modelar e são as principais causas das variações no consumo voluntário observadas nesse estágio fisiológico de bovinos.

Com base nisso, objetivou-se neste estudo:

1. Avaliar o crescimento e desenvolvimento de tecidos maternos e fetais durante a gestação em vacas zebuínas;
2. Desenvolver um grupo de equações e relações para ajuste de peso corporal em vacas gestantes e não gestantes, ampliando as opções para a pesquisa na área de gado de corte;
3. Avaliar os efeitos da gestação e do nível alimentar sobre o consumo, digestibilidade e eficiência de produção de compostos nitrogenados microbinos em vacas Nelore;

4. Descrever equações e relações para predição das exigências líquidas, metabolizáveis e dietéticas de energia e proteína para vacas Nelore adultas, gestantes e não gestantes.

LITERATURA CITADA

AGRICULTURAL AND FOOD RESEARCH COUNCIL - AFRC. **Energy and protein requeriments of ruminants.** Wallingford: CAB International, 1993. 159p.

AGRICULTURAL RESEARCH COUNCIL - ARC. **The nutrient requirements of ruminants livestock**. London: CAB International, 1980. 351p.

ANUALPEC. **Anuário da Pecuária Brasileira.** São Paulo: Angra FNP Pesquisas, 2011.

BATTAGLIA, F.C.; MESCHIA, G. Fetal nutrition. **Annual Review of Nutrition**, v.8, p.43–61, 1988.

BAUMAN, D.E.; CURRIE, W.B. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. **Journal of Dairy Science**, v.63, p.1514–1529, 1980.

BELL, A. W. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. **Journal of Animal Science**, v.73, n.9, p.2804-2819, 1995.

BELL, A. W.; FERRELL, C. L.; FREETLY, H. C. Pregnancy and Fetal Metabolism. In: DIJKSTRA, J.; FORBES, J.M.; FRANCE, J.(Ed). **Quantitative aspects of ruminant digestion and metabolism**. 2.ed. Oxfordshire: CAB International, 2005, p.523-550.

BELL, A.W. Pregnancy and fetal metabolism. In: FORBES, J.M. e FRANCE, J. (eds) **Quantitative Aspects of Ruminant Digestion and Metabolism**. CAB International, Wallingford, pp. 405–431, 1993.

BELL, A.W.; EHRHARDT, R. A. Regulation of macronutrient partitioning between maternal and conceptus tissues in the pregnant ruminant. In: CRONJÉ, J.S.(Ed). **Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction**. 1.ed. New York: CAB International, 2000, p.275-293.

BELL, A.W.; KENNAUGH, J.M.; BATTAGLIA, F.C. et al. Uptake of amino acids and ammonia at mid-gestation by the fetal lamb. **Quarterly Journal of Experimental Physiology**, v.74, p.635–643, 1989.

CANO, J.G. The INRA systems of nutritional requirements of cattle. In: SIMPÓSIO INTERNACIONAL SOBRE EXIGÊNCIAS NUTRICIONAIS DE RUMINANTES, 1., 1995, Viçosa. **Anais…** Viçosa: JARD: Universidade Federal de Viçosa, [1995].

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANAZATION – CSIRO. **Nutrient Requirements of Domesticated Ruminants**. CSIRO Publishing. Collingwood. 2007. 296p.

FERRELL, C.L.; GARRETT, W.N.; HINMAN, N. Estimation of Body Composition in Pregnant and Non-Pregnant Heifers. **Journal of Animal Science**, v.42, n.5, p.1158-1166, 1976b.

FERRELL, C.L.; GARRETT, W.N.; HINMAN, N. et al. Energy Utilization by Pregnant and Non-Pregnant Heifers. **Journal of Animal Science**, v.42, n.4, p.937-950, 1976c.

FERRELL, C.L.; GARRETT, W.N.; HINMAN, N. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. **Journal of Animal Science**, v.42, n.6, p.1477-1489, 1976a.

FISHER, D.S.; BURNS, J.C.; POND, K.R. Modeling ad libitum DM intake by ruminants as regulated by distension and chemostatic feedbacks. **Journal of Theoretical Biology**, v.126, p.407-418, 1987.

FORBES, J.M. A model of the short-term control of feeding in the ruminant: effects of changing animal or feed characteristics. **Appetite**, v.1, p.21-41, 1980.

FORBES, J.M. The effects of sex hormones, pregnancy, and lactation on digestion, metabolism and voluntary intake. In: MILLIGAN, L.P., GROVUM, W.L. e DOBSON, A. (eds) **Control of Digestion and Metabolism in Ruminants**. Prentice- Hall, Englewood Cliffs, New Jersey pp. 420–435, 1986.

FREETLY, H.C.; FERRELL, C.L. Net flux of glucose, lactate, volatile fatty acids, and nitrogen metabolites across the portal-drained viscera and liver of pregnant ewes. **Journal of Animal Science**, v.76, p.3133–3145, 1998.

FREETLY, H.C.; FERRELL, C.L. Net flux of non-esterified fatty acids, cholesterol, triacylglycerol, and glycerol across the portal-drained viscera and liver of pregnant ewes. **Journal of Animal Science**, v.78, p.1380–1388, 2000.

HAMMOND, J. Animal breeding in relation to nutrition and environmental conditions. **Biological Reviews**, v.22, p.195–213, 1947.

HOLZMAN, I.R.; LEMONS, J.A.; MESCHIA, G. et al. Ammonia production by the pregnant uterus. **Proceedings of the Society for Experimental Biology and Medicine**, v.156, p.27–30, 1977.

IBGE – Instituto Brasileiro de Geografía e Estatística. **Censo Agropecuário 2011**. Disponível em: <www.ibge.gov.br/home/estatistica/economia/agropecuaria> Acesso em: 09/11/2012.

INGVARTSEN, K.L.; ANDERSEN, B.B. Integration of metabolism and intake regulation: A review focusing on periparturient animals. **Journal of Dairy Science**, v.83, p.1573-1597, 2000.

INGVARTSEN, K.L.; ANDERSEN, H.R.; FOLDAGER, J. Effect of Sex and Pregnancy on Feed Intake Capacity of Growing Cattle. **Acta Agriculturae Scandinavica, Section A - Animal Science**, v.42, n.1, p.40-46, 1992.

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE – INRA. **Alimentation des bovins, ovins et caprins.** In: JARRIGE, R. (ed); Quae, Paris, 2007. 330p.

MESCHIA, G.; BATTAGLIA, F.C.; HAY, W.W. et al. Utilization of substrates by the ovine placenta in vivo. **Federation Proceedings**, v.39, p.245–249, 1980.

NATIONAL RESEARCH COUNCIL – NRC. **Nutrients requirements of dairy cattle.** 7.ed. National Academic Press. Washington, D.C., 2001. 381p.

PRIOR, R.L.; LASTER, D.B. Development of the bovine fetus. **Journal of Animal Science**, v.48, n.6, p.1546-1553, 1979.

RITCHIE, R.W. The optimum cow – what criteria must she meet? In: RESEARCH SYMPOSIUM AND ANNUAL MEETING, 27., 1995, Kansas City. **Proceedings…** Kansas City: Beef Improvement Federation, p.126-145, 1995.

VASCONCELOS, J.L.M; MENEGHETTI, M. Sincronização de ovulação como estratégia par aumentar a eficiência reprodutiva de fêmeas bovinas, em larga escala. In: SIMPÓSIO DE PRODUÇÃO DE GADO DE CORTE, 5, Viçosa, MG. **Anais...** Viçosa: UFV, p.529-541, 2006.

OBS.: Os capítulos 1, 3 e 4 apresentados a seguir foram escritos de acordo com as normas do Journal of Animal Science. O capítulo 2 foi escrito de acordo com as normas da revista PLoS ONE.

CHAPTER 1

Growth and development of cow, udder and gravid uterus of Nellore cows during pregnancy

ABSTRACT: Forty-nine nonlactating multiparous Nellore cows (32 pregnant and 17 non-pregnant) with an initial BW of 451 ± 10 kg were used in an experiment to evaluate the growth and development of maternal and fetal organs and tissues during pregnancy in zebu cows and the effect of feeding level on tissue development. Cows were segregated into 2 groups according to level of feeding; either **HIGH** (*ad libitum*) or **LOW** (restricted feeding 1.2 times maintenance according to the NRC). The 32 pregnant cows were separated into 4 groups of 8 cows each and harvested at 136, 189, 239 and 269 d of pregnancy for tissue collection and analysis. HIGH-fed cows had greater BW gain ($P < 0.01$), final BW ($P < 0.01$) and body condition score ($P < 0.01$). In early gestation, LOW-fed and HIGH-fed cows had similar accretion of fat in the empty body free of pregnancy components (**EBW_{np}**), however, with the increase in gestational age, the daily rates of fat gain were greater in HIGH-fed cows than those in LOW-fed cows. The BW gain in pregnant LOW-fed cows was mostly fat while HIGH-fed cows accumulated also other types of tissues. LOW-fed cows had lower proportion of organs and viscera (139 vs 159 g/kg of EBW_{np}, $P < 0.01$). The interaction between feeding level and time of gestation ($P = 0.03$) showed that the gravid uterus was greater in HIGH-fed cows only in late gestation, although there were no differences in chemical composition and energy content of gravid uterus due to feeding level ($P > 0.10$). HIGH-fed cows increased the weight of fresh udder after 239 d of gestation ($P < 0.10$) while LOW-fed cows did not present significant udder increase due to pregnancy ($P > 0.10$).

LOW-fed cows had proportionally (g/kg of EBW_{np}) lower liver, visceral fat and mesentery (*P* < 0.01) and greater heart (*P* < 0.10) than HIGH-fed cows. The proportional weights of rumen-reticulum, omasum and total stomach were affected (*P* ≤ 0.05) by pregnancy and were lower at 269 d than in early gestation. HIGH-fed cows had greater fetuses at 269 d of gestation than LOW-fed cows. Fetuses from HIGH-fed cows had greater carcasses (*P* = 0.03) but did not differ from fetuses from LOW-fed cows in organs and viscera (*P* = 0.27). Placenta weight was greater in HIGH-fed cows (*P* < 0.01) and the relationship between placenta and fetus weight was different between feeding levels (*P* < 0.01). The weights of the gravid uterus and fetus, as well as their contents of energy and N, were lower in this study compared to previous studies with *Bos taurus* cattle.

KEYWORDS: beef cattle, *Bos indicus*, fetal programming, fetus, homeorhesis, maternal nutrition

RESUMO: Quarenta e nove vacas Nelore (32 gestantes e 17 não gestantes) com peso médio inicial de 451 ± 10 kg foram usadas num experimento para avaliar o crescimento e desenvolvimento de tecidos maternos e fetais durante a gestação em vacas zebuínas. As vacas foram divididas em dois grupos de acordo com o nível alimentar, que foi ALTO (ad libitum) ou BAIXO (alimentação restrita a 1,2 vezes a mantença, segundo o NRC). As 32 vacas gestantes foram separadas em 4 grupos com 8 vacas em cada e abatidas aos 136, 189, 239 e 269 dias de gestação para coletas de tecidos e análises. Vacas do alto nível alimentar tiveram maior ganho de peso (P<0,01), peso final (P<0,01) e escore de condição corporal (P<0,01). No início da gestação, as vacas dos dois grupos alimentares tiveram similar acréscimo de gordura no peso de corpo vazio isento de componentes relativos à gestação ($PCVZ_{np}$). Entretanto, com o aumento da gestação, as taxas diárias de ganho de gordura foram maiores nas vacas de alto nível alimentar do que nas do nível baixo. O ganho de peso obtido por vacas do nível alimentar baixo foi praticamente apenas gordura, enquanto que vacas do alto nível alimentar acumularam também outros tipos de tecidos. Vacas do baixo nível alimentar tiveram menor proporção de órgãos e víscera (139 vs 159 g/kg de $PCVZ_{np}$, P<0.01). A interação entre o nível alimentar e o tempo de gestação (P=0,03) mostrou que o útero grávido foi maior nas vacas do nível alimentar alto no final da gestação, embora não fossem observadas diferenças significativas na composição química e conteúdo de energia do útero grávido em função do nível alimentar (P>0,10). Vacas do nível alimentar alto aumentaram o peso do úbere após 239 dias de gestação (P<0,10) enquanto que vacas do nível alimentar baixo não apresentaram aumento significativo. Vacas do menor nível alimentar tiveram proporcionalmente menores pesos de fígado, gordura visceral e mesentério (P<0,01) e maior coração (P<0,10) do que vacas do nível alimentar alto. Os pesos proporcionais do rúmen-retículo e dos estômagos foram afetados ($P<0,05$) pela gestação e foram menores aos 269 dias de gestação do que no início da gestação. Vacas do nível alimentar alto tiveram fetos mais pesados aos 269 dias de gestação do que vacas do nível alimentar baixo. Os fetos das vacas do maior nível alimentar tiveram maiores carcaças ($P=0,03$), mas não diferiram dos fetos das vacas do menor nível alimentar no peso de órgãos e vísceras ($P=0,27$). O peso da placenta foi maior em vacas do maior nível alimentar ($P<0,01$) e a relação entre peso da placenta e do feto foi diferente entre os níveis alimentares ($P<0,01$). Os pesos do útero grávido e fetos, como também seus conteúdos de energia e nitrogênio, foram menores neste estudo do que em estudos prévios realizados com gado *Bos taurus*.

Palavras-chave: *Bos indicus*, feto, gado de corte, homeorrese, nutrição materna, programação fetal

INTRODUCTION

Pregnancy is the most complex physiological stage of a beef cow's life. Several challenges occur in the normal physiology status of the cow to support calf formation. These modifications include changes in the size and composition of the maternal tissues and organs and growth of the tissues that support pregnancy and lactation such as the gravid uterus and mammary gland. The dynamic of these changes generally follows an exponential model (Jakobsen et al., 1957; Ferrell et al., 1976a; Prior and Laster, 1979) with an increase of the variation in fetal weight and gravid uterus as the pregnancy nears its end (Ferrell et al., 1976a; Silvey and Haydock, 1978; Bell et al., 1995).

Factors such as genotype (Anthony et al., 1986; Ferrell, 1991; Bellows et al., 1993), number of fetuses (Gregory et al., 1990; Ferrell and Reynolds, 1992), nutrition (Freetly et al., 2000; Greenwood et al., 2002; Meyer et al., 2010) and temperature all affect development of pregnancy. Nutrition affects the development of the progeny during the pregnancy and after birth (Wu et al., 2006; Vonnahme, 2007).

The knowledge about the dynamic of the modifications that occur in the cow and its progeny because the level of intake and at different stages of pregnancy may lead to challenges with regard to nutritional management during gestation.

Most studies about pre-natal growth in cattle were developed with *Bos taurus* animals. However, *Bos indicus* and *B. taurus* differ in the physiology of growth and development (Frisch and Vercoe, 1977; Jenkins and Ferrell, 2004; Sartori et al., 2010). Unfortunately, information regarding *B. indicus* pre-natal growth are rare and usually limited to birth weight (Souza and Ramos, 1995; Lobato et al., 1998; Forni et al., 2009) or estimation of fetal measures by ultrasound during the pregnancy (Bergamaschi et al., 2004). Thus, the objective was to evaluate the growth and development of maternal and fetal organs and tissues during pregnancy in zebu cows and the effect of feeding level on tissue development.

MATERIALS AND METHODS

This study was conducted at the Universidade Federal de Viçosa (Viçosa, MG, Brazil) following the standard procedures for humane animal care and handling according to the university's guidelines.

Animals

Forty-nine nonlactating multiparous Nellore cows with an initial BW of 451 ± 10 kg, age ranging from 3.8 to 7.9 years and body condition score of 4.4 ± 0.2 (1 to 9 scale) were obtained from the Universidade Federal de Viçosa herd and commercial farms. Before the experiment, cows were allowed to graze forage (*Brachiaria decumbens* Stapf.) receiving a mineral and vitamins supplement. Animals were treated for internal and external parasites by the administration of ivermectin (Ivomec, Merial, Duluth, GA).

Thirty-two cows were separated at random and hand mated to 5 Nellore bulls (6-7 cows per bull) during a 50-d mating period. At the beginning of the mating period a hormonal induction of the estrous cycle was performed using an injection of GnRH agonist followed 7 d later by an injection of $PGF_{2\alpha}$ (Thatcher et al., 1989; Wolfenson et al., 1994). Mating observation was performed by 2 observers 3-times per d (0600 to 0800, 1200 to 1400, and 1800 to 2000 h) and 25 d after mating ultrasound was used to confirm pregnancy. Day of mating was considered the d 0 of pregnancy.

From the initial 49 cows, 12 cows were separated at random and assigned to the non-pregnant group and the 5 remaining cows were designated as the baseline group and harvested.

During the mating period cows were kept in drylot pens (1000 m²) with 6-7 cows and 1 bull in each pen. Cattle were fed a diet containing fresh sugar cane (45% on DM basis), elephant grass (*Pennisetum purpureum*, 45% on DM basis) and concentrate (10% on DM basis, 20% CP). The concentrate portion of the diet was composed of sorghum meal, urea and mineral mixture. The non-pregnant and baseline cows were kept in a separate pen and fed the same diet. Before the beginning of the experiment cows were moved to the experimental pens and allowed to adapt to the electronic head gate system (Kloppen Soluções Tecnológicas, Pirassununga, SP, Brazil) and the experimental diet for 3 wk.

Diet and management

Cows were housed in pens (48 m², 6 cows per pen) with a concrete floor, 15 m² of covered area, and *ad libitum* access to fresh water. Feed intake was measured individually using an electronic head gate system. Bred cows were at 47 ± 3 d of gestation when the feeding trial started.

All cows were fed the same diet twice daily (0700 and 1500 h) and segregated into 2 groups according to level of feeding, either **HIGH** (*ad libitum*) or **LOW** (restricted feeding 1.2 times maintenance according to the NRC, 2000). Sixteen pregnant and 5 non-pregnant cows were fed HIGH level and 16 pregnant and 7 non-pregnant cows were fed the LOW-diet. The restricted feeding was estimated so that 1.2 times maintenance sustained pregnancy and the HIGH-fed allowed some maternal tissue storage.

The diet consisted of corn silage, corn meal, soybean meal, urea and mineral mixture (Table 1). The feed supplied and the orts were recorded daily. Samples of corn silage and orts were taken daily and composited each week for analyses. Samples of the corn meal and soybean meal were taken each time the concentrate was mixed.

Every 28 d cows were weighed in the morning (0700 h, before feeding) to obtain the BW, and the next day, at the same time, after 16 h of fasting a shrunk BW (**SBW**) measure was taken. The cows had ad libitum access to water during these times. Thus, the body gain (**BG**, kg/d) and the shrunk body gain (**SBG**, kg/d) were calculated.

The 32 pregnant cows were separated at random into 4 groups of 8 cows each (4 cows per each feeding level) and harvested at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. The non-pregnant cows were harvested at different times of the experiment (85 to 216 days of feeding control) to keep them in the experiment for a similar length of time as the pregnant cows. The baseline cows were harvested on d 0 of the experiment after the end of the adaptation period and were used to estimate the initial body chemical composition of the cows in the experiment.

Animal harvest and sampling

Pre-harvest animal care and handling procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). Before harvest, feed was withheld from animals for 16 h, but they had *ad libitum* access to water the entire time. Harvest was performed by using captive bolt stunning and exsanguination. The euthanasia of fetuses was achieved according American Veterinary Medical Association Guidelines (AVMA, 2013).

At harvest, udders were removed along with the portion of hide that covered the udder. Udders were weighed, ground, and samples were taken for chemical analyses.

The uterus were severed at the cervix and collected for analyses. The gravid uterus was weighed and dissected into the fetus, uterus, ovaries, and the placenta and fetal fluids (allantoic and amniotic fluids). All of the gravid uterus components were individually weighed and the weights of the fetal fluids were calculated by difference. The uterus and ovaries were ground with the placenta and a sample was collected for chemical composition analyses. Samples of fetal fluids were also collected for chemical analyses.

Biometric measures of the fetuses were collected similar to the procedures of Lyne (1960) and Ferrell et al. (1976a). The following measures were recorded: body length, thoracic circumference, height at shoulder, height at rump, cranial eye circumference and cranial neck circumference (Figure 1).

Fetuses were dissected initially into head, legs, carcass (containing the hide), organs and viscera, and the reproductive apparatus (udder and uterus and ovaries or penis and testicles). Organs and viscera were separated and individually weighed. The weights of heart, liver, kidneys, lungs, spleen, pancreas and digestive tract (rumen plus reticulum, omasum, abomasum, small intestine, and large intestine) were recorded. After that, all of the fetal body were regrouped and ground in a bowl cutter (Refere Inox, Chapecó, SC, Brazil) for a composited chemical composition sample of the whole fetus.

The cow's whole body was initially segregated into head, legs, hide, organs and viscera, blood and carcass (lean, fat and bone) and then weighed. Organs and viscera were separated and individually washed and weighed. The weights of heart, liver, kidneys, lungs, diaphragm, spleen, visceral fat, mesentery and digestive tract (rumen plus reticulum, omasum, abomasums, small intestine, large intestine and mesenteric fat) were recorded. The grouping of all whole body components after the wash of the digestive tract constituted the pregnant empty BW (**EBW_p**, including udder and gravid uterus of pregnant cows) or the empty BW (**EBW_{np}**, for non-pregnant cows). The EBW_{np} of pregnant cows was estimated according Gionbelli (2013). After weighing, the head, legs, hide, organs and viscera were ground. One composite sample of all of non-carcass compounds (head, members, blood, hide, organs and viscera) was taken for chemical composition analyses.

The carcasses were weighed and chilled in a cold chamber (4 ºC) for 24 h. After chilling, the carcasses were weighed and separated into bone and soft tissue (lean and fat). All carcass tissues were ground, grouped and sampled for chemical composition analyses.

The soft tissue components were ground using a bowl cutter and the rigid tissues were ground initially in an industrial grinder (TOL10, Lunasa, Araguari,MG, Brazil), and subsequently in a bowl cutter. The homogenization and the grouping to make the carcass and non-carcass composite samples also were performed using a bowl cutter. All samples were stored frozen (-80 ºC) until laboratory chemical analyses.

Chemical analyses

All samples were lyophilized using a freeze dryer. The feed and orts samples were ground using a Willey mill (TE-650, Tecnal, Piracicaba, SP, Brazil) to pass through a 1 mm screen. The samples of animal tissues were frozen-powdered using liquid nitrogen and a blender. The DM was collected by oven drying at 100 ºC for 18 hours (Method 934.01; AOAC, 2000). The ash content was determined by 2-h incineration process in a 600 ºC muffle furnace (Method 942.05; AOAC, 2000). The CP content was calculated based on the N content multiplied by the 6.25 factor. The N content was calculated by the Kjeldahl procedure (Method 920.87; AOAC, 2000). Evaluation of the ether extract (**EE**) content was performed with a XT15 extractor (Ankom, Macedon, NY) using petroleum ether, according to the American Oil Chemist's Society method (Am5-04; AOCS, 2009). Gross energy was measured using an adiabatic bomb calorimeter (C5001, IKA-Werke Co, Staufen, Germany).

Statistics analysis

Means comparisons. The response variables were analyzed using the GLM procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC) according to the following model:

$$Y_{ijk} = \mu + F_i + G_j + (F \times G)_{ij} + e_{ijk}$$

where F_j is the jth level of the fixed effect of feeding level, G_k is the k_{th} level of the fixed effect of days of gestation and e_{ijk} is the random error.

This model was used to all studied response variables besides cow's body parts, organs and viscera. In addition to all the effects in the model, the fixed covariate effect of cow's BW was included to adjust the values. Least squares means were estimated for all effects and compared using Fischers protected least significant difference test at α = 0.10.

Regression models. When appropriate, regression models were fitted to the data to explain better the growth and development of the gravid uterus and its components. The procedures REG and GLM of SAS were used to estimate the regression parameters of linear functions. Parameters for nonlinear functions were estimated using the Gauss-Newton method in NLIN procedure of SAS version 9.2. Residual biases were tested by regressing model residuals on predicted values and testing if the mean residual differed from zero.

The basic mathematical model used to estimate the dynamics of gravid uterus and its components in function of time of pregnancy was of the logistic form, similar to that of Koong et al. (1975):

$$W = W_0 \times e^{(\beta_1 + \beta_2 \times t + \beta_3 \times L) \times t} \qquad [1]$$

where t is the day of gestation, L is the level of feeding (g of DM/kg of SBW), W_0 is the amount of component on day 0 of gestation and W is the amount of component on day t of gestation.

When partial weights were compared to estimated weight at parturition, a gestation length of 290 days was used based on average Nellore cow gestation length (Cavalcante et al., 2001; Rocha et al., 2005).

Model evaluations. Parameters of non-linear functions were estimated for HIGH-fed, LOW-fed cows and pooled functions when appropriate. For each function, a likelihood ratio test was calculated to test whether estimation of parameters specific to each feeding level significantly improved fit of the data relative to estimation of parameters from a pooled data set, ignoring the feeding level. The test statistic was as follows:

$$F = \frac{(RSS_P - RSS_H - RSS_L)/(Rdf_P - Rdf_H - Rdf_L)}{(RSS_H + RSS_L)/(Rdf_H + Rdf_L)}$$

where RSS represents residual sums of squares and Rdf denotes residual degrees of freedom; the subscripts P, H and L indicated pooled model, HIGH and LOW-fed cows, respectively. A *P*-value for the F distribution was used to evaluate if the single model was inappropriate and that the relationship between traits differs between feeding level. The value of $P = 0.10$ was adopted as critical level of probability for occurrence of Type I error.

RESULTS AND DISCUSSION

The average DMI was 16.0 ± 2.0 and 10.8 ± 1.5 g/kg of SBW/d for cows fed HIGH and LOW level of intake, respectively.

Cows body change and chemical composition

Body change. At the beginning of the experiment (47 d of gestation) cows had similar SBW (Table 2). As a result of the feeding levels applied SBW was different ($P < 0.01$) among feeding level groups throughout the gestational period and also for the non-pregnant cows. This difference in BW was because the difference of SBG ($P < 0.01$, Table 2) among feeding level groups.

Even when subtracting the weight gain relative to the growth of the pregnancy (real shrunk body gain, **rSBG**) HIGH-fed cows had greater body gain (0.73 vs 0.15 kg/d) than LOW-fed cows. Cows of LOW feeding level reached the expected gain (0.15 kg/d) to support the pregnancy with the minimum of maternal body tissues gain. This value of weight gain did not cause changes ($P > 0.10$) in the final BW of LOW-fed cows throughout the gestation periods and when compared to the non-pregnant cows (**rfSBW**, Table 2). An increase in final BW without the gravid uterus was observed for HIGH-fed cows with increased gestation time which indicates an accumulation of body reserves.

Among HIGH-fed cows, non-pregnant cows had greater rSBG ($P < 0.10$) than pregnant cows as from 189 d of pregnancy indicating a reduction in the body reserves due to the need for transfer of nutrients to the development of the gravid uterus.

The HIGH-fed cows had greater BCS than LOW-fed cows (*P* < 0.01, Table 2) and cows in late gestation had greater BCS than cows in early gestation. However, when the BCS was expressed as points of BCS earned every 100 d of experiment (BCSG), no differences were observed (*P* > 0.10) among gestational times and among non-pregnant cows. The differences on BCSG values noted among HIGH-fed and LOW-fed cows were proportional to the values of rSBG and were about 4 times greater in HIGH-fed cows.

Nitrogen. There were significant (*P* < 0.10) accretion of N on cow's body from 239 d of gestation (Table 3) but it was not significant (*P* = 0.27) when evaluated in the EBW_{np}. This finding suggests that although pregnant cows have stored body reserves during gestation (Table 2), these reserves were not comprised of protein, although a greater amount of N was observed in HIGH-fed than LOW-fed cows. The HIGH-fed cows gained an average of 2 kg of N (Table 4) while in LOW-fed cows the gain of N was negligible (average of 0.16 kg). The gain of N in HIGH-fed cows was similar among gestational times evaluated and among pregnant and non-pregnant cows. On the other hand, a greater rate of N gain (*P* < 0.10) was observed at early gestation compared to whole gestation or non-pregnant cows. In the pregnant LOW-fed cows the gain of N was negligible, but there was N gain (6.22 g/d) for non-pregnant cows. These results suggest that changes in the metabolism of protein deposition may have occurred in the cow's body due to the gestation. Although LOW-fed cows had accumulated small body reserves during gestation, these reserves were not in the form of protein.

The low gain rates of N were compatible with the physiological maturity of the animals used in this experiment. Adult multiparous mature cows already reached the peak of maturity and usually do not gain large amounts of N. It is suggested that the increase of N in HIGH-fed cows during the pregnancy occurred as a consequence of increase of organs size and skeletal muscle hypertrophy. The gain rate of N in this experiment was lower than those observed in growing animals (Jennings et al., 2011; Bonilha et al., 2013).

Fat. Body fat increased (in the non-pregnant empty body) only in HIGH-fed cows ($P < 0.10$; Table 3). The LOW-fed cows accumulated about half of the amount of fat throughout the experiment than the HIGH-fed cows (23.1 vs 51.8 kg, Table 4). The gestation seems to cause a positive effect in rates of fat deposition in LOW-fed pregnant cows when compared to LOW-fed non-pregnant cows. In early gestation, LOW-fed and HIGH-fed cows had no differences in accretion of fat in the empty body; however, with the increase in gestational age, the daily rates of fat gain were greater in HIGH-fed cows than those in LOW-fed cows. As presented in Table 4 body gain in LOW-fed cows was mostly fat while HIGH-fed cows accumulated also other types of tissues.

Ash. HIGH-fed cows had less proportion of ash ($P = 0.05$) in the composition of EBW_{np} than LOW-fed cows (Table 3), probably due to an effect of dilution caused by the higher proportion of fat. The HIGH-fed cows had average body gain of ash of 1.88 kg (Table 4) while in LOW-fed cows the gain of ash was near to zero, being positive for non-pregnant cows and slightly negative in pregnant LOW-fed cows, which might indicate a need to mobilize tissue to meet the requirements of the gravid uterus. In HIGH-fed cows the rate of daily gain of ash was greater ($P < 0.10$) in early gestation and decrease after 189 d of gestation, likely indicating a greater need of nutritional support for the growing gravid uterus.

Water. The increase of body water as a result of gestation was significant ($P < 0.10$) only when the cows empty body was evaluated as a whole, which also includes the gravid uterus (Table 3). When the gravid uterus was excluded from the evaluation, the amount of water present in the body of the cows was not different ($P = 0.23$) with increasing pregnancy. This is likely a result of the gravid uterus having a high proportion of water (Table 3), which could be up to 44 kg of water in the cow's body. The HIGH-fed cows gained 35.5 kg of water throughout the experiment while the LOW-fed cows gained a negligible amount of water (Table 4) suggesting no gain of lean tissue in these group of cows. The gain of water throughout the gestation period in HIGH-fed cows was proportional to the gain of N, which might be explained by the increase of lean tissue.

It seems to be a priority in the accumulation of tissues throughout gestation in beef cows. It was observed in this study (Table 3 and Table 4) that in LOW-fed cows the lower amount of observed gain (Table 2) was related mostly to body fat deposition, while in HIGH-fed cows there was also accretion of other type of tissues, e.g. bone and lean tissue. This observation indicates a priority order of deposition of tissues inverse to that normally observed in growing animals. Specifically, there is a greater proportion of deposition of lean tissue in cattle with low rates of gain and deposition of lean tissue and fat in cattle with high rates of gain.

Energy. The increase in the amounts of energy observed in this study was related to the increase in the amounts of body fat. The amount of energy retained in the gravid uterus is low significant up to 239 d of gestation (Table 3). The energy retained in the gravid uterus represents respectively 0.2, 0.5, 1.5 and 2.0 percent of the total energy in the body of cows at 136, 189, 239 and 269 d of gestation. As a result, the net energy requirements for pregnancy in cattle is low, but significant when expressed in metabolizable energy, because there is great energy expenditure related to the synthesis and maintenance of tissues related to the pregnancy (Ferrell et al., 1976a). The concentration of energy in the gravid uterus (Mcal/kg) observed in this study in relation to the concentration of energy in the cow's non-pregnant body was of 1:9.9, 1:6.6, 1:4.8 and 1:4.6 at 136, 189, 239 and 269 d of gestation, respectively.

Enhancement of body energy concentration in pregnant cows was observed with the increase of gestational age (Table 3) when the non-pregnant empty body was evaluated. HIGH-fed cows had greater daily rate of energy gain than LOW-fed cows (4.13 vs 1.89 Mcal/d, Table 4). However, when the average concentration of energy of gain was evaluated, the estimated values of rEBG were 12.6 and 5.4 Mcal/kg for LOW-fed and HIGH-fed cows, respectively. The energy concentration in the gain of LOW-fed cows is greater than the energetic coefficient of fat (9.39; Blaxter and Rook, 1953) because the variations on body composition along the experiment (loss of water and fat gain, Table 4).

It is noteworthy that LOW-fed cows showed only fat body gain which may be related to the nutrient requirements for gestation. The growth of the pregnant uterus or conceptus (fetus, placenta, fetal fluids, fetal membranes and supporting tissues of the uterus) in the last third of gestation requires maternal supply of glucose and amino acids in large amounts (Bell, 1995). Some models of metabolic patterns for pregnancy in cows and sheep (Bell, 1993; Bell, 1995; Bell et al., 2005) predict that in both species during the last third of gestation, 35 to 40% of fetal energy is provided as glucose and lactate and 55% is provided as amino acids. Most of the remaining 5 to 10% is provided by acetate, which is very low compared to its abundance and relative importance to the maternal metabolic system. The capacity of the placenta to transport long chain non-esterified fatty acids (NEFA) and ketone bodies is rather limited (Bell et al., 2005), avoiding fetal access to substrates derived from mobilization of maternal body fat and thereby restricting the ability of the fetus to grow at the expense of maternal energy reserves. Almost all the N acquired by the fetus is in the form of amino acids, although a small umbilical uptake of ammonia is observed from the intermediate stage of pregnancy (Holzman et al., 1977; Bell et al., 1989). Thus, the mobilization of amino acids that are precursors of gluconeogenesis and also to meet the supply of amino acids of the conceptus is often required. Therefore, an insufficient amounts of CP in the diet leads to loss of skeletal muscle tissue in the carcass of the dam, as observed by McNeill et al. (1997).

Cows body parts, organs and viscera

Carcass and non-carcass tissues. Carcass characteristics and meat quality of the cows used in this experiment were presented by Duarte et al. (2013b). Cows of HIGH feeding level had carcass and non-carcass tissues (head, hide, feet, blood, organs and viscera) heavier ($P < 0.01$) than LOW-fed cows (Table 5). Although it has been observed an increase on cow carcass weight as a function of gestation time, this increase was not observed ($P = 0.20$) in the non-carcass tissues. When expressed as proportion of EBW_{np} (Table 6) carcass and non-carcass tissues did not differ ($P = 0.11$ and $P = 0.17$, respectively) as a function of time of gestation ($P = 0.35$ and P = 0.33, respectively). The dissection of non-carcass tissues showed that LOW-fed cows had higher proportion of head, feet and hide ($P < 0.03$) and lower proportion of organs and viscera (139 vs 159 g/kg of EBW_{np}, P < 0.01, Table 6) than HIGH-fed cows. The increase in the proportions of head, feet and hide may be explained by a dilution effect because the growth of these tissues is probably not affected by feeding level, and LOW-fed cows had lower body size than HIGH-fed cows (Table 2). The reduction of the proportion of organs and viscera may represent an attempt to save energy by LOW-fed cows to support pregnancy. There is evidence that dams reduce visceral oxygen consumption during periods of food restriction (Freetly et al., 1995) indicating that the results of this study are consistent with this fact.

Uterus. The gravid uterus increased the size about 73 times compared to a non-pregnant uterus (47 vs 0.64 kg, Table 5). The interaction between feeding level and time of gestation ($P = 0.03$) showed that the gravid uterus was greater ($P < 0.10$) in HIGH-fed cows only in late gestation (51.7 vs 42.4 kg at 269 d of gestation, Table 5). The results obtained in this study were similar to that observed in sheep when ewes where fed either ad libitum or at maintenance, and significant differences in the gravid uterus weight were observed only in late pregnancy (Rattray et al., 1974). In a study using cattle as a model, Ferrell et al. (1976a) did not observed differences in the development of gravid uterus during gestation when Hereford heifers were fed at either 150 or 215 kcal/ME/$EBW^{0.75}$/d.

The adjust of a logistic model (Eq. [1]) was efficient to describe the growth of gravid uterus in function of time of gestation (Table 7). Greater gravid uterus weight in HIGH-fed cows at 269 d of gestation suggested ($P = 0.10$) that the use of a single model to describe the growth of the gravid uterus as function of d of gestation in HIGH and LOW-fed cows may not be suitable. The average gravid uterus weight estimated at parturition (using models of Table 7) was 55.0 kg (67.9 and 45.0 kg for HIGH and LOW-fed, respectively), which represents an average of 9.6 % of SBW of a cow in the same period. The estimated gravid uterus weight at 270 d of gestation in this study was 47.0 kg, which is 19% lower than the gravid uterus weight estimated for the same period in Hereford heifers (58.0 kg, Ferrell et al., 1976a). This is consistent with lower average birth weight of *B. indicus* cattle (Holland and Odde, 1992).

Although the feeding level has caused significant differences in the whole weight of the gravid uterus during late gestation in Nellore cows, there were no significant differences in chemical composition of gravid uterus between feeding levels (*P* > 0.41; Table 3). HIGH-fed cows had more fat and water in the gravid uterus than LOW-fed cows at 269 d of gestation. However there were no differences (*P* = 0.47) in the N and energy content between the two groups, similar to what was previously reported by Ferrell et al. (1976a). This fact suggests that there were no differences in the net N and energy requirements for pregnancy in Nellore cows caused by the feeding level.

The amounts of N and energy in the gravid uterus observed in this study were lower than those present in the gravid uterus of Hereford heifers (Ferrell et al., 1976a). The amounts of N were [this study vs Ferrell et al. (proportion %)] 0.071 vs 0.073 (97%), 0.18 vs 0.24 (76%), 0.56 vs 0.65 (85%) and 0.79 vs 1.14 (69%) at 136, 189, 239 and 269 d of gestation, respectively. The amounts of energy (Mcal) were 2.73 vs 3.39 (81%), 7.2 vs 11.7 (62%), 22.9 vs 32.6 (70%) and 32.2 vs 56.6 (57%) at 136, 189, 239 and 269 d of gestation, respectively. These results suggest that the net requirements of N and energy for pregnancy are lower in *B. indicus* than in *B. taurus* beef cattle.

Udder. There was interaction (*P* = 0.07) between the feeding level and time of gestation for the whole weight of the cow's udder (Table 5). HIGH-fed cows increased the weight of udder as from 239 d of gestation, while LOW-fed cows did not present significant increase. The amount of dry matter in the udder showed increase only in late gestation (269 d) for HIGH-fed cows, while for LOW-fed cows there was no significant increase (Table 8). The amounts of N in udder increase in late gestation and were greater in HIGH-fed cows than LOW-fed cows. When the amounts of N were expressed in proportion of fresh weight of udder (g/kg), there were no effect (*P* = 0.15) of feeding level and the results also showed an increase (*P* < 0.01) in the proportion of N along the gestation. There was an interaction (*P* = 0.03) between the feeding level and the time of pregnancy in amount of fat of fresh udder. The amount of fat in udder of HIGH-fed cows was greater ($P < 0.10$) at 269 d of gestation than at 189 d of gestation but similar to what was observed in cows at other times of gestation evaluated. A decrease in the amount of fat ($P < 0.10$) was observed in LOW-fed cows after 239 d of gestation. Proportionally, the amount of fat in the udder decrease with the increase of time of gestation, with reduction more accentuated in LOW-fed cows (interaction, $P = 0.03$). The amount of ash was greater ($P = 0.02$) in the udder of HIGH-fed cows and has also increased as the time of gestation increased (Table 8). This enhancement was also observed when the quantities were presented as proportion of udder fresh weight. The amount of water in the fresh udder increased ($P < 0.01$) during gestation but it was not affected by feeding level ($P = 0.57$).

When presented proportionally the content of water in the gravid uterus was lower ($P < 0.01$) in HIGH-fed cows compared to LOW-fed cows, inversely to what was observed with fat (Table 8). An interaction between feeding level and time of gestation ($P = 0.02$) showed that the difference in the content of water in the udder among the feeding levels occur after 239 d of gestation. No differences were observed ($P = 0.35$) in the content of energy (Mcal) in the udder of cows as a function of time of gestation. However an interaction between feeding level and time of gestation showed a tendency of increase in the amount of energy in the udder of HIGH-fed cows and reduction in LOW-fed cows as from 239 d of gestation (Table 8). The energy concentration in the udder of HIGH-fed cows was lower during gestation comparing to the non-pregnant cows ($P < 0.10$) and did not differ among the times of gestation. In the LOW-fed cows was observed decrease of the energy concentration at 239 and 269 d of gestation comparing to the empty LOW-fed cows.

The results of weight and chemical composition of the udder of the cows in this study suggest that HIGH-fed cows had greater amount of fat and consequently energy in the udder than LOW-fed cows. Unlike what was observed by Ferrell et al. (1976a), the greater amount of fat in the udder of HIGH-fed cows was not excessive to the point of causing detriment to the formation of secretory tissue as the amount of N and ash content in the udder of HIGH-fed cows observed in late gestation was constant and not-decreasing. It can be noted that Ferrell et al. (1976a) used heifers while in this study mature cows were used, suggesting that the effect of high amounts of fat in the udder may cause detriment to the formation of secretory tissue only in heifers.

In this study it was observed the exchange of fat N, ash and water as the time of gestation advanced, suggesting the formation of parenchymal tissue secreting milk, that was more evident in the late gestation (239 and 269 d of gestation). The fat concentration decrease from 736 g/kg in non-pregnant cows to 485 g/kg at 269 d of gestation, while the water concentration increase from 105 g/kg (non-pregnant cows) to 323 g/kg (269 d of gestation). These differences indicate the development or hypertrophy of milk secretory tissue in the late gestation. Although there were no significant effect of feeding level in the N and ash concentration ($P = 0.15$ and $P = 0.29$, respectively), HIGH-fed cows had greater total amounts of N and ash in the udder than LOW-fed cows in the late pregnancy (Table 8). This result suggests that HIGH-fed cows would produce more milk or colostrum than LOW-fed cows.

Swanson et al. (2008) reported a reduction in the weight of the mammary gland, colostrums production and total IgG in restricted-fed ewes (60% of requirements) comparing to the control (100% of requirements) and overfeeding (140% of requirements) treatments. Hammer et al. (2011) observed that lambs from restricted ewes having increased blood IgG 24 h after birth comparing with lambs from controlled and high feeding level ewes, when all lambs received the same amount of the same artificial colostrum. This result suggests that lambs from restricted ewes could be more efficient to absorb IgG, as result of the lower colostrum production from their dams. In the present study the fetuses were dissected and a complete evaluation of the development of the gastrointestinal tract was performed (Duarte et al., 2013a) showing that the fetuses from LOW-fed cows had greater length of the small intestine as well as larger intestinal villi. This is consistent with the lowest amount of ash and N observed in udders of LOW-fed cows, suggesting that the explanation for the results observed by Duarte et al. (2013a) is related to the same fact that occurred in studies of Swanson et al. (2008) and Hammer et al. (2011).

The exchange of fat per secretory tissue, even with increased weight of the udder did not reflect in increased amounts of fat (P = 0.27) and energy (P = 0.35) in the udder as the gestation increase. These data suggest that there is no increase in the nutrient requirements of gestation in function of the increase of udder weight, similar to what has been previously observed by Ferrell et al. (1976a). Unlike this, the amounts of N and ash were rising as a function of time of gestation (P < 0.01), indicating that the addition of N and ash in the udder should be accounted when estimating the nutritional requirements for gestation.

Cows organs and viscera. HIGH-fed cows had greater liver, kidneys, lungs, diaphragm, spleen, visceral fat, mesentery and gastrointestinal tract (P < 0.01) than LOW-fed cows (Table 5). No differences were observed in the weights of heart (P = 0.28) between the feeding levels, although proportionally (g/kg of EBW_{np}) the heart of LOW-fed cows was smaller than those from HIGH-fed cows (P < 0.01). Although some studies have suggested an increase in the systemic blood flow and volume as the gestation progresses (Rosenfeld, 1977; Freetly and Ferrell, 1997) the observed changes in the mass of blood due to gestation remains unclear. The blood mass was greater (P < 0.10) at 269 d of gestation compared to 189 and 239 d, but did not differ (P > 0.10) of blood mass at 136 d (Table 5). Proportionally to the EBW_{np} of cows, the content of blood at 136 d of gestation was greater ($P < 0.10$) than in non-pregnant cows and the cows from 189 and 239 d of gestation and did not differ ($P > 0.10$) from 269 d cows (Table 6).

In several studies with ruminant animals have been observed changes in the relative proportion of visceral organs to body mass caused by the feeding level in pregnant (Scheaffer et al., 2004; Reed et al., 2007; Carlson et al., 2009; Caton et al., 2009; Meyer et al., 2010) and growing animals (Burrin et al., 1990). In general, the increase in the weight of organs was proportional to the increase in the ingestion of food, similar to that was observed in the current study, except for liver, visceral fat and mesentery, that were proportionally (g/kg of EBW_{np}) lower ($P < 0.01$) in LOW-fed cows. Meyer et al. (2010) also observed low proportional weight of liver in feed-restricted cows, unlike of Carlson et al. (2009) in which ewes that were restricted during midgestation had greater proportional liver mass than control ewes near term. The liver is an organ that presents high oxygen consumption and energy expenditure (Eisemann and Nienaber, 1990). It can be suggested that the liver, heart and kidneys answered for about 37% of the fasting heat production in cattle and that only the liver is responsible for about 22.5% (Ferrell et al., 1976b). In the current study, LOW-fed cows had a liver 39% smaller than HIGH-fed cows (3.99 vs 5.54, Table 5), which would represent a heat production about 9% lower (22.5% × 39%) only due to the liver size. Visceral fat and mesentery represent largely energy stored in the form of fat, which expresses low storage of energy as visceral fat by LOW-fed cows.

With regard to the effects of gestation on the mass of visceral organs, in the early study of Ferrell et al. (1976a) there were no differences in offal weight or composition between pregnant and non-pregnant heifers. Later studies showed changes in visceral organs mass caused by pregnancy (Scheaffer et al., 2001; Scheaffer et al., 2004; Caton et al., 2009; Meyer et al., 2010), although the results are partly contradictory.

In the current study pregnant cows decrease the mass of liver compared to the non-pregnant cows (*P* < 0.10), although the liver did not change the weight between the periods of gestation (*P* > 0.10, Table 5). As BW increased over time, a reduction in the proportional weight of liver (g/kg of EBW_{np}) as the gestation time increased was observed (*P* < 0.01, Table 6). In previous studies the weight of liver was greater in the late gestation than in mid-gestation in heifers (Scheaffer et al., 2001), ewe lambs (Caton et al., 2009), and mature ewes (Scheaffer et al., 2004) or equal between the two periods (for non-restricted cows, Meyer et al., 2010), being similar to the results observed in the current study. Freetly and Ferrell (1997) also reported increase of hepatic oxygen consumption as day of gestation increased in mature ewes. It suggested that visceral organ weight may increase as gestation progresses because increasing nutrient flux from increased fetal demand (Freetly and Ferrell, 1998). It could be hypothesized that because the smaller size and lower energy content of the gravid uterus in this study compared to those reported by Ferrell et al. (1976a), *B. indicus* cows have lower nutrient requirements for pregnancy and not need or need less increasing the size of the organs than *B. taurus* cattle or ewes.

There was an interaction (*P* = 0.01) between the time of pregnancy and feeding level for visceral fat. The amount of visceral fat was greater (*P* < 0.10) in the HIGH-fed cows than in LOW-fed cows and between 189 and 239 d of gestation, being equal in early and late gestation (136 and 269 d of gestation, respectively). HIGH-fed cows increased the amount of visceral fat as from 136 d of gestation, while LOW-fed cows increased the amount of visceral fat only at the late gestation (269 d).

Gastrointestinal tract dissection. The crude weights of all portions of gastrointestinal tract were lower in LOW-fed cows ($P < 0.03$, Table 5) than in HIGH-fed cows, as function of the greater body size and processing of greater amount of food. When expressed proportionally (g/kg of EBW_{np}, Table 6) there was no effect of feeding level on the portions of gastrointestinal tract ($P > 0.26$). However, it was observed reduction in the weight of rumen-reticulum ($P < 0.01$) and omasum ($P = 0.05$) due to increase in gestation time. This reduction is possibly caused by limited space. It has been suggested that the compression of the rumen by gravid uterus and visceral fat reduce the dry matter intake in late gestation in ruminants (Forbes, 2007). Forbes (1968) observed drastic reduction in ruminal volume when ewes were slaughtered and immediately frozen and then sawn in cross-sections, at various stages of gestation. Forbes (1969) observed a negative relationship between ruminal volume at the slaughter and the volume of compressive content (gravid uterus plus abdominal fat) in ewes. In the current study cows reduce the rumen-reticulum weight in 6.9% (7.43 vs 6.95 kg, altought not statistically significant, Table 5) comparing cows with 136 d of gestation and 269 d cows. At the same time, the non-pregnant BW increased 7.0% resulting in a proportional reduction (g of rumen/kg of EBW_{np}) of 18.8% (Table 6). Considering the rumen volume as a sphere, the rumen wall with a thickness of 8 mm (Roozbahani et al., 2013) and a density of 1:1 (weight:volume), the reduction of 8.18% on the rumen weight is a reduction of approximately 37% in volume (80.0 to 58.3 litres) between 136 and 239 d of gestation.

Cows in the current study showed no differences in mass and proportional weight along the gestation, similar Meyer et al. (2010) and in contrast to other studies where the mass of small intestine was lower in cows that suffered restriction in the middle and late gestation (Scheaffer et al., 2004; Reed et al., 2007; Carlson et al., 2009) or only in late gestation (Carlson et al., 2009), although it is suggested that the mass of the small intestine is responsive to nutrient restriction at the late or during all gestation (Meyer et al., 2010).

Gravid uterus development

Because the few number of studies in the literature, data on the development of the gravid uterus components were presented in detail (Table 9), with values dismembered for feeding level even when the interaction among feeding level and gestation time was not significant ($P > 0.10$). Nonlinear models of logistic type were fitted to the data of gravid uterus, fetus, uterus, placenta and fetal fluids (Table 7).

Estimated fetal, uterus, placenta and fetal fluids at parturition were obtained by extrapolation of the appropriate regression to 290 days of gestation. The values obtained were 28.8, 9.03, 1.53 and 10.5 kg for the fetus, uterus, placenta and fetal fluids. The weights of these tissues as a percentage of estimated weight at birth are shown in various days of gestation in Figure 4. These data indicate that placental development precedes the development of the uterus, which in turn precedes the development of the fetus. These results support the information presented previously by some authors (Dawes, 1968; Ferrell et al., 1976a), that fetal development is dependent on a previous development of the uterus and placenta, which are required to supply nutrients for developing fetus.

Fetus weight. The fresh weight of fetuses from HIGH-fed cows was greater ($P < 0.10$) than those from LOW-fed cows only at 269 d of gestation ($P = 0.01$ for interaction nutrition × gestation, Table 9). The average weights of fetus in the current experiment were [this study vs Ferrell et al., 1976a (proportion %)] 1.50 vs 1.67 (90%), 6.33 vs 7.45 (85%), 19.9 vs 21.2 (94%) and 27.1 vs 33.6 (81%) for cows with 136, 189, 239 and 269 d of gestation. The average fetal weight estimated at parturition in percentage of dam weight was 5.1% in HIGH-fed cows and 5.5% in LOW-fed cows, compared to an average of 7% for *B. taurus* cattle (Robbins and Robbins, 1979). These values corroborate data from the literature affirming that *B. indicus* cattle have calves with lower birth weight than *B. Taurus* cattle (Andersen and Plum, 1965; Holland and Odde, 1992). It should be noted also that the variation in the ratio of weights indicates that there is a difference in the rate of fetal development between *B. indicus* and *B. taurus* cattle, when data from the current study were compared with data from Ferrell et al. (1976a).

It can be hypothesize that the lower weight of fetuses from LOW-fed cows at 269 d of gestation is because the lower availability of nutrients for growth and maintenance of the gravid uterus. The effects of maternal nutrition on calf weight at birth have been found controversial. Some studies suggested that birth weight is only significantly affected by the level of maternal nutrition in situations of severe nutritional deficiencies or excessive amount of energy (Holland and Odde, 1992).

Knowingly gender affects birth weight in cattle with males being about 4-8% heavier than females (Holland and Odde, 1992). Although in the current study the sex of fetuses was not verified before the slaughter, there was at least one male or female in each of the two feeding groups in each gestational time evaluated. The effects of sex and cow initial BW were not significant in the model used to evaluate the fetal (P = 0.46 and 0.22 for sex and cow initial BW, respectively) and gravid uterus weight (P = 0.32 and 0.18 for sex and cow initial BW, respectively).

Uterus weight. The gravid uterus weight estimated at parturition (9.03 kg, using pooled equation from Table 7) was average 15.8 times greater than the uterus of non-pregnant cows (0.57 kg). In the first two thirds of pregnancy the growing of the uterus occurs due to hormonal factor that causes increase in uterine muscular hypertrophy. At the end of gestation the uterus ceases growth due to hormonal factors and starts to grow by stretching (Assali, 1968).

The weight of empty uterus increase as gestation increase (Table 9 and Figure 2) but decrease in proportion of the gravid uterus weight (g/kg) indicating that the growth of this tissue precedes the fetal weight (Figure 4). The proportional size of the empty uterus was also slightly greater (P = 0.07) in LOW-fed cows than in HIGH-fed cows (Table 9).

Placenta weight. The placenta of HIGH-fed cows was greater (P < 0.01) than the placenta of LOW-fed cows throughout the gestation (Table 9 and Figure 2). When evaluated as proportion of the gravid uterus (g/kg) the placenta of HIGH-fed cows was greater (P < 0.10) than the placenta of LOW-fed cows at 139 and 189 d of gestation and did not differ (P > 0.10) at 239 and 269 d of gestation. Two hypothesis can be considered from this fact. The placenta of LOW-fed cows was more efficient to play its role and was lower because the need of lower energy expenditure or; the lower placenta of LOW-fed cows may have produced fetuses with less potential of skeletal muscle development, that were lower than fetuses from HIGH-fed cows at 269 d of gestation.

The relationship between the placenta and fetal weight observed in the current study was different (P < 0.01) among the feeding levels and is presented in Figure 5. Although the placenta of LOW-fed cows was lower, the fetuses from these cows were not different from fetuses from HIGH-fed cows at 136, 189 and 239 d of gestation. This is related to the fact that the efficiency of the placenta was different between the feeding levels.

The placenta plays a key role in fetal growth (Belkacemi et al., 2010) and its size is high correlated with the fetal size (Anthony et al., 1986). Limited growth of the placenta is considered almost universally as the main determinant of intrauterine growth retardation in mammals (Bassett, 1991). The small placental size results in a reduction in uterine blood flow to the placenta, so placental transport and delivery of both oxygen and glucose to the fetus are reduced (Owens et al., 1986; Bassett, 1991). However, in the study of Owens et al. (1987), in which endometrial caruncles were removed from sheep (caruncle sheep) before mating, which restricted placental growth in the subsequent pregnancy, only half the fetuses of caruncle sheep were small or growth retarded, with the remainder normal in size, even with placental growth restriction.

Fetal fluids weight. The amount of fetal fluids was not affected (P = 0.81) by the feeding level, similarly to that was observed in previous studies with sheep (Rattray et al., 1974) and cattle (Ferrell et al., 1976a). As the gestational age increases the amount of fetal fluids increased rapidly at the early gestation, did not have increase between 136 and 189 d of gestation and increased again at 239 and 269 d of gestation, similar to that observed by Ferrell el al. (1976a) although in that study the increased amount of fetal fluids ceased between 189 and 237 d of gestation. Unlike the current study, Prior and Laster (1979) observed linear increase of fetal fluids as the gestation increases. An exponential model was the best fit to the data in the current study (Table 7) and a graphical representation is shown in Figure 2.

Fetus development and chemical composition

Fetus body compounds. The difference observed in fetal weight between the feeding levels at 269 d of gestation was mostly related to the carcass weight of fetuses from HIHG-fed cow (Table 10). While the weight of the carcass with hide was 29% greater, the weights of organs and viscera, head and feet were 16%, 14% and 8% greater, respectively, in fetuses from HIGH-fed cows compared to fetus from LOW-fed cows. This suggests that differences in fetal weight caused by the dam feeding level may occur more pronounced in skeletal muscle and bone development of the fetus.

Recent studies suggest that nutrient deficiency in ruminant animals can cause reduction of muscle fiber number and muscle mass, and consequently affecting performance and meat quality of progeny (Du et al., 2010). The fetal stage is crucial for skeletal muscle development because there is no increase in muscle fibers numbers after birth (Du et al., 2011).

Fetus organs and viscera. No differences on fresh weight (g) or proportional weight (g/kg) of heart, lungs, kidneys, liver, spleen, pancreas and gastrointestinal tract due to feeding level were observed (P > 0.10, Table 10 and Table 11). All evaluated fetal organs had their fresh (g) and proportional weight (except gastrointestinal tract) affected by the gestational age (P < 0.03, Table 10 and Table 11).

All the investigations reviewed by Bassett (1991) seem to indicate that small placental size limits fetal growth. However, when the effects on the relative growth of individual tissues and organs were examined, marked differential effects, not simply a consequence of the allometric nature of growth, are found. In the current study although LOW-fed cows had lower placentas (Table 9), there were no significant differences in the organs and viscera between fetuses from cows of the different feeding levels. From all of fetus body compounds and organs and viscera, the feeding level, and consequently the weight of placenta, is related only with differences on fetal carcass weight (P = 0.03, Table 10). When body parts and organs and viscera were presented as proportion of fetus weight (Table 11) no differences in the dynamic of individual tissues were found as function of the feeding level.

The fetal organs and viscera evaluated had some reduction in proportional weight during pregnancy (except gastrointestinal tract), but the points of decrease or stabilization varied between organs. The dynamic of the fetal heart, lungs, kidneys, liver, spleen and pancreas in function of gestational age are presented in Table 12. Functions were generated based on Eq. [1]. Based on generated functions, the proportional weights of organs were also presented as proportion of fetal BW (g/kg) at 145 and 290 d of gestation (half gestation and at parturition) and compared with the same proportional weights in a mature cow (Table 12). The day of gestation when the weight of which organ is equal to 50% of the predicted weight at parturition is also presented in Table 12. The organs weight and the daily rate of gain of each organ as percentage of predicted weight at parturition are presented in Figure 6 and Figure 7, respectively.

Based on this set of information can be noted that the fetal organs had different growth dynamics as function of gestational age. The kidneys develop faster than other organs reaching half of its estimated size at birth at 199 d of gestation (Table 12) and a peak growth rate at around 210 d of gestation (Figure 7). It can be assumed that this is because even in early pregnancy the renal function is already similar to adulthood .

The lung is the organ that has delayed development because is not functional in the fetus. With birth, the function of gas exchange is transferred from the placenta to the lungs, and it is probably because this that fetal lung growth rate reaches its peak only near parturition. Organs with functions of the same group as the liver and pancreas had similar growth rate curves. The spleen is the only organ that has assessed proportionally lower weight at birth than in the adult animal (Table 12). The heart is the only of the evaluated organs that had a higher proportion in relation to fetal weight at birth than in mid-pregnancy.

Biometric measures of fetus. Except cranial neck circumference there were no differences on fetal biometric measures in function of feeding level (Table 13). Fetus from HIGH-fed cows had greater cranial neck circumference (cm) than fetus from LOW-fed cows at 269 d of gestation. All the biometric measures increased throughout the gestation but at a different rate than fetus weight. The biometric measures of distance showed larger increase than the circumference measures as the pregnancy increases.

Fetal biometric measures are highly correlated with fetal age and weight. Data from Lyne (1960) and Ferrell et al. (1976a) suggest linear relationships between day of gestation and linear measures of fetal size beyond 120 d of gestation. Before 120 d of gestation curvilinear relationships are suggested (Ferrell et al., 1976a). In the current study linear equations fit well to the data from linear measures of fetal size. However, non-linear equations of the type $y=ax^b$ showed better fit (lower Akaike information criterion; Akaike, 1974) and were therefore used. Equations to predict the fetal age and weight from the biometric measures were constructed (Table 14). These equations may have very useful to predict fetal age and weight using ultrasound. However, these regressions do not predict fetal ages accurately in early gestation, thus should not be extrapolated beyond the scope of these data (130 to 272 d of gestation).

Data from the current study was compared to data from previous studies (Lyne, 1960; Ferrell et al., 1976a) to estimate fetus body length (also called as straight crown-rump). The fetus body length (cm) was 25.6, 31.0, 29.9 and 31.4 cm at 135 d of gestation and 78.2, 89.9, 76.2 and 90.8 cm at 270 d of gestation using data from Ferrell et al. (1976a, *B. taurus*), Lyne (1960) for *B. taurus*, Lyne (1960) for *B. taurus* × *B.*

indicus and the current study (*B. indicus*), respectively. Based on these results Nellore fetuses seems to have equal or greater body length than *B. taurus* fetuses at the same fetal age even with lower weight.

Fetus chemical composition. Fetus from HIGH-fed cows had more water (g) than fetus from LOW-fed cows at 269 d of gestation (Table 15). No significant effects of feeding level were observed on fetal amounts of N, fat, ash and energy. The greater amount of water in fetus from HIGH-fed cows at 269 d of gestation seems to be related with the hypothesis that the fetuses from HIGH-fed cows were greater at 269 d of gestation by having greater development of skeletal muscle. Although not significant the amount of N in fetuses of HIGH-fed cows was 14% greater than in fetuses from LOW-fed cows at 269 d of gestation. The non-significance of feeding level for the energy content (P = 0.61) indicates that although some differences were observed in the size of fetuses between feeding levels, there are no differences in nutrient requirements for fetal growth in cows fed with different levels of feeding.

When evaluated as proportion of fetus weight (g/kg) the interaction between feeding level and gestational age was significant for the contents of N, ash, water and energy. Fetus from LOW-fed cows had greater proportion of N and energy at 269 d of gestation and ash at 239 and 269 d of gestation than HIGH-fed cows (Table 15). Fetuses from HIGH-fed cows had lower proportion of water at 189 d of gestation and greater proportion of water at 239 and 269 d of gestation than fetuses from LOW-fed cows. These results seem agree with the hypothesis of greater amount of skeletal lean tissue in fetuses from HIGH-fed cows.

Fetuses from cows of the current study showed lower concentrations of N and energy than fetuses from study of Ferrell et al. (1976a). The proportions of N (g/kg) were 10.9 and 12.2 (current study *vs* Ferrell’s study) at 136 and 21.1 and 26.1 at 269 d of gestation. The proportions of energy (Mcal/kg) were 0.42 and 0.55 (current study *vs* Ferrell's study) at 136 and 0.91 and 1.34 at 269 d of gestation.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model indentification. IEEE transactions on Automatic Control AC-19: 716-723.

Andersen, H., and M. Plum. 1965. Gestation Length and Birth Weight in Cattle and Buffaloes: A Review1,2. J. Dairy. Sci. 48: 1224-1235.

Anthony, R. V. et al. 1986. Fetal GRowth of Beef Calves. II. Effect of Sire on Prenatal Development of the Calf and Related Placental Characteristics. J. Anim. Sci. 62: 1375-1387.

AOAC. 2000. Official Methods of Analysis of AOAC International. 17th ed. Association of Official Analytical Chemists, Arlington, VA.

AOCS. 2009. Official Methods and Recommended Practices of the AOCS. 6th ed. American Oil Chemists' Society, Denver, CO.

Assali, N. S. 1968. Biology of Gestation: The fetus and neonate. Academic Press.

AVMA. 2013. Guidelines for the Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association, Schaumburg.

Bassett, J. M. 1991. Current perspectives on placental development and its integration with fetal growth. Proceedings of the Nutrition Society 50: 311-319.

Belkacemi, L., D. M. Nelson, M. Desai, and M. G. Ross. 2010. Maternal Undernutrition Influences Placental-Fetal Development. Biology of Reproduction 83: 325-331.

Bell, A. W. 1993. Pregnancy and fetal metabolism. In: J. M. Forbes and J. France (eds.) Quantitative Aspects of Ruminant Digestion and Metabolism. p 405-431. CAB International, Wallingford.

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., 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., J. M. Kennaugh, F. C. Battaglia, and G. Meschia. 1989. Uptake of amino acids and ammonia at mid-gestation by the fetal lamb. Quarterly Journal of Experimental Physiology 74: 635-643.

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.

Bellows, R. A., R. B. Staigmiller, L. E. Orme, R. E. Short, and B. W. Knapp. 1993. Effects of sire and dam on late-pregnancy conceptus and hormone traits in beef cattle. J. Anim. Sci. 71: 714-723.

Bergamaschi, M. A. C. M., W. R. R. Vicente, R. T. Barbosa, J. A. Marques, and A. R. Freitas. 2004. Effect of grazing system on fetal development in Nellore cattle. Theriogenology 61: 1237-1245.

Blaxter, K. L., and J. A. F. Rook. 1953. The heat of combustion of the tissues of cattle in relation to their chemical composition. British Journal of Nutrition 7: 83-91.

Bonilha, E. F. M. et al. 2013. Body chemical composition of Nellore bulls with different residual feed intakes. J. Anim. Sci. 91: 3457-3464.

Brasil. 1997. Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal [*Regulation of Industrial and Sanitary Inspection of Animal Products*]. Ministério da Agricultura Pecuária e Abastecimento, Brasília, DF.

Burrin, D. G., C. L. Ferrell, R. A. Britton, and M. Bauer. 1990. Level of nutrition and visceral organ size and metabolic activity in sheep. British Journal of Nutrition 64: 439-448.

Carlson, D. B. et al. 2009. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes. J. Anim. Sci. 87: 669-680.

Caton, J. S. et al. 2009. Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J. Anim. Sci. 87: 222-235.

Cavalcante, F. A., R. Martins Filho, C. C. Campello, R. N. B. Lobo, and G. A. Martins. 2001. Período de gestação em rebanho Nelore na Amazônia Oriental. Revista Brasileira de Zootecnia 30: 1451-1455.

Dawes, G. S. 1968. Foetal and neonatal physiology: a comparative study of the changes at birth. Year Book Medical Publishers.

Du, M. et al. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88: E51-E60.

Du, M. et al. 2011. Fetal muscle development, mesenchymal multipotent cell differentiation, and associated signaling pathways. J. Anim. Sci. 89: 583-590.

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

Duarte, M. S. et al. 2013b. Effects of pregnancy and feeding level on carcass and meat quality traits of Nellore cows. Meat Science 94: 139-144.

Eisemann, J. H., and J. A. Nienaber. 1990. Tissue and whole-body oxygen uptake in fed and fasted steers. British Journal of Nutrition 64: 399-411.

Ferrell, C. L. 1991. Maternal and fetal influences on uterine and conceptus development in the cow: II. Blood flow and nutrient flux. J. Anim. Sci. 69: 1954-1965.

Ferrell, C. L., W. N. Garrett, and N. Hinman. 1976a. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. J. Anim. Sci. 42: 1477-1489.

Ferrell, C. L., W. N. Garrett, N. Hinman, and G. Grichting. 1976b. Energy Utilization by Pregnant and Non-Pregnant Heifers. J. Anim. Sci. 42: 937-950.

Ferrell, C. L., and L. P. Reynolds. 1992. Uterine and umbilical blood flows and net nutrient uptake by fetuses and uteroplacental tissues of cows gravid with either single or twin fetuses. J. Anim. Sci. 70: 426-433.

Forbes, J. M. 1968. The physical relationships of the abdominal organs in the pregnant ewe. The Journal of Agricultural Science 70: 171-177.

Forbes, J. M. 1969. The effect of pregnancy and fatness on the volume of rumen contents in the ewe. The Journal of Agricultural Science 72: 119-121.

Forbes, J. M. 2007. Voluntary Food Intake and Diet Selection in Farm Animals. 2nd ed. CABI Publishing, Wallingford, UK.

Forni, S. et al. 2009. Comparison of different nonlinear functions to describe Nelore cattle growth. J. Anim. Sci. 87: 496-506.

Freetly, H. C., and C. L. Ferrell. 1997. Oxygen consumption by and blood flow across the portal-drained viscera and liver of pregnant ewes. J. Anim. Sci. 75: 1950-1955.

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., C. L. Ferrell, and T. G. Jenkins. 2000. Timing of realimentation of mature cows that were feed-restricted during pregnancy influences calf birth weights and growth rates. J. Anim. Sci. 78: 2790-2796.

Freetly, H. C., C. L. Ferrell, T. G. Jenkins, and A. L. Goetsch. 1995. Visceral oxygen consumption during chronic feed restriction and realimentation in sheep. J Anim Sci 73: 843-852.

Frisch, J. E., and J. E. Vercoe. 1977. Food intake, eating rate, weight gains, metabolic rate and efficiency of feed utilization in *Bos taurus* and *Bos indicus* crossbred cattle. Animal Production 25: 343-358.

Gionbelli, M. P. 2013. NUTRIENT REQUIREMENTS AND QUANTITATIVE ASPECTS OF GROWTH, DEVELOPMENT AND DIGESTION OF PREGNANT AND NON-PREGNANT NELLORE COWS. PhD Thesis, Universidade Federal de Viçosa, Viçosa.

Greenwood, P. L. et al. 2002. Fetal growth capacity influences nutritional status of Hereford cows during pregnancy. Anim. Prod. Aust. 24: 304.

Gregory, K. E. et al. 1990. Twinning in cattle: III. Effects of twinning on dystocia, reproductive traits, calf survival, calf growth and cow productivity. J. Anim. Sci. 68: 3133-3144.

Hammer, C. J. et al. 2011. Effects of maternal selenium supply and plane of nutrition during gestation on passive transfer of immunity and health in neonatal lambs. J. Anim. Sci. 89: 3690-3698.

Holland, M. D., and K. G. Odde. 1992. Factors affecting calf birth weight: A review. Theriogenology 38: 769-798.

Holzman, I. R., J. A. Lemons, and G. Meschia. 1977. Ammonia production by the pregnant uterus. Proceedings of the Society for Experimental Biology and Medicine 156: 27-30.

Jakobsen, P. E., P. H. Sorensen, and H. Larsen. 1957. Energy Investigations as Related to Fetus Formation in Cattle. Acta Agriculturae Scandinavica 7: 103-112.

Jenkins, T. G., and C. L. Ferrell. 2004. Preweaning efficiency for mature cows of breed crosses from tropically adapted Bos indicus and Bos taurus and unadapted Bos taurus breeds. J Anim Sci 82: 1876-1881.

Jennings, J. S. et al. 2011. Circulating ghrelin and leptin concentrations and growth hormone secretagogue receptor abundance in liver, muscle, and adipose tissue of beef cattle exhibiting differences in composition of gain. J. Anim. Sci. 89: 3954-3972.

Koong, L. J., W. N. Garrett, and P. V. Rattray. 1975. A Description of the Dynamics of Fetal Growth in Sheep. J. Anim. Sci. 41: 1065-1068.

Lobato, J. F. P., R. L. D. Zanotta Júnior, and O. A. Pereira Neto. 1998. Effects of pre and post-partum diets of primiparous beef cows on the growth of their calves. Revista Brasileira de Zootecnia 27: 863-867.

Lyne, A. G. 1960. Pre-natal growth of cattle. Proceedings of Australian Society of Animal Production 3: 153-161.

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.

Meyer, A. M. et al. 2010. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. J. Anim. Sci. 88: 2410-2424.

NRC. 2000. Nutrient Requirements of Beef Cattle. updated 7th ed. National Academy Press, Washington, DC.

Owens, J. A., J. Falconer, and J. S. Robinson. 1986. Effect of restriction of placental growth on umbilical and uterine blood flows. American Journal of Physiology 250: R427-434.

Owens, J. A., J. Falconer, and J. S. Robinson. 1987. Effect of restriction of placental growth on fetal and utero-placental metabolism. Journal of Developmental Physiology 9: 225-238.

Prior, R. L., and D. B. Laster. 1979. Development of the bovine fetus. J. Anim. Sci. 48: 1546-1553.

Rattray, P. V., W. N. Garrett, N. E. East, and N. Hinman. 1974. Growth, Development and Composition of the Ovine Conceptus and Mammary Gland During Pregnancy. J. Anim. Sci. 38: 613-626.

Reed, J. J. et al. 2007. Effects of selenium supply and dietary restriction on maternal and fetal body weight, visceral organ mass and cellularity estimates, and jejunal vascularity in pregnant ewe lambs. J. Anim. Sci. 85: 2721-2733.

Robbins, C. T., and B. L. Robbins. 1979. Fetal and neonatal growth patterns and maternal reproductive effort in ungulates and subungulates. The American Naturalist 114: 101-116.

Rocha, J. C. M. C., H. Tonhati, M. M. Alencar, and R. B. Lôbo. 2005. Genetic parameters estimates for gestation length in beef cattle. Arq. Bras. Med. Vet. Zootec. 57: 784-791.

Roozbahani, M. A. et al. 2013. Ultrasonographic Thicknesses of Ruminal and Abdominal Wall in High Yielding Holstein Dairy Cows. Life Science Journal 10: 93-96.

Rosenfeld, C. R. 1977. Distribution of cardiac output in ovine pregnancy. American Journal of Physiology - Heart and Circulatory Physiology 232: H231-H235.

Sartori, R. et al. 2010. Physiological differences and inplications to reproductive management of Bos taurus and Bos indicus cattle in a tropical environment. Society of Reproduction and Fertility supplement 67: 357-375.

Scheaffer, A. N., J. S. Caton, M. L. Bauer, and L. P. Reynolds. 2001. Influence of pregnancy on body weight, ruminal characteristics, and visceral organ mass in beef heifers. J. Anim. Sci. 79: 2481-2490.

Scheaffer, A. N., J. S. Caton, D. A. Redmer, and L. P. Reynolds. 2004. The effect of dietary restriction, pregnancy, and fetal type in different ewe types on fetal weight, maternal body weight, and visceral organ mass in ewes. J. Anim. Sci. 82: 1826-1838.

Silvey, M. W., and K. P. Haydock. 1978. A note on live-weight adjustment for pregnancy in cows. Animal Production 27: 113-116.

Souza, J. C., and A. A. Ramos. 1995. Efeitos de fatores genéticos e do meio sobre os pesos de bovinos da raça Nelore. Revista Brasileira de Zootecnia 24: 164-172.

Swanson, T. J. et al. 2008. Effects of gestational plane of nutrition and selenium supplementation on mammary development and colostrum quality in pregnant ewe lambs. J. Anim. Sci. 86: 2415-2423.

Thatcher, W. W., K. L. Macmillan, P. J. Hansen, and M. Drost. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology 31: 149-164.

Vonnahme, K. A. 2007. Nutrition during gestation and feral programming. In: Proceedings of the Range Beef Cow Symposium, XX, Fort Collins, CO. p 10 p.

Wolfenson, D., W. W. Thatcher, J. D. Savio, L. Badinga, and M. C. Lucy. 1994. The effect of a GnRH analogue on the dynamics of follicular development and synchronization of estrus in lactating cyclic dairy cows. Theriogenology 42: 633-644.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. BOARD-INVITED REVIEW: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84: 2316.

Table 1. Ingredients and chemical composition of the diet

Item	Silage	Concentrate	Diet
Ingredient , % of DM			
Corn Silage	100.0	-	84.3
Ground corn	-	54.6	8.5
Soybean meal	-	33.0	5.1
Urea	-	7.3	1.2
Sodium chloride	-	2.1	0.37
Ammonium sulfate	-	1.5	0.25
Dicalcium phosphate	-	1.3	0.23
Microminerals mixture[1]	-	0.17	0.028
Analyzed composition[2], %			
DM	28.0	89.2	37.6
OM	94.7	92.8	94.4
CP	7.8	44.1	13.5
EE	2.9	2.5	2.8
NDF_{ap}	45.8	8.2	39.9
iNDF	20.8	0.65	17.6
NDIN	38.2	7.0	11.4
NFC	38.2	53.0	40.6
TDN	-	-	66.6
GE (Mcal/kg)	3.82	3.49	3.77

[1]Zinc sulfate (56.3 %), manganese sulfate (26.2 %), copper sulfate (16.8 %), potassium iodate (0.37 %), cobalt sulfate (0.23 %) and sodium selenite (0.10 %).
[2]NDF_{ap} = neutral detergent fiber corrected to ash and protein, iNDF = indigestible neutral detergent fiber and NFC = non fibrous carbohydrates.

Table 2. Body change, daily gain and body condition score of pregnant and non-pregnant Nellore cows in function of days of gestation and feeding level

Item[1]	Day of gestation					Feeding level[2]		P-value[3]		
	Empty (n = 12)	136 (n = 8)	189 (n = 8)	239 (n = 8)	269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
iBW[nc], kg	449 ± 20	463 ± 24	434 ± 24	464 ± 24	437 ± 24	450 ± 15	449 ± 15	0.96	0.84	0.81
iSBW[nc], kg	435 ± 20	454 ± 24	422 ± 24	448 ± 24	423 ± 24	436 ± 15	437 ± 14	0.98	0.83	0.81
fSBW[nc], kg	497 ± 20	508 ± 26	494 ± 26	555 ± 26	551 ± 26	563 ± 16	480 ± 16	<0.01	0.25	0.39
fSBW, kg	501 ± 7	492 ± 9	509 ± 9	545 ± 9	565 ± 9	564 ± 6	481 ± 5	<0.01	<0.01	0.08
High	535[de] ± 11	515[cd] ± 13	554[e] ± 13	591[f] ± 13	623[g] ± 13	-	-	-	-	-
Low	467[a] ± 10	468[ab] ± 13	464[a] ± 13	498[bc] ± 13	506[c] ± 13	-	-	-	-	-
rfSBW, kg	501 ± 7	484 ± 9	495 ± 9	509 ± 9	518 ± 9	543 ± 5	460 ± 5	<0.01	<0.01	0.07
High	535[bc] ± 11	508[b] ± 12	540[c] ± 12	558[cd] ± 12	572[d] ± 12	-	-	-	-	-
Low	467[a] ± 10	459[a] ± 12	450[a] ± 12	461[a] ± 12	464[a] ± 12	-	-	-	-	-
SBG, kg/day	0.54 ± 0.05	0.62 ± 0.06	0.48 ± 0.06	0.59 ± 0.06	0.58 ± 0.06	0.86 ± 0.04	0.26 ± 0.04	<0.01	0.60	0.98
rSBG, kg/day	0.54[c] ± 0.05	0.53[bc] ± 0.06	0.38[a] ± 0.06	0.40[ab] ± 0.06	0.37[a] ± 0.06	0.73 ± 0.04	0.15 ± 0.04	<0.01	0.07	0.95
iBCS[nc]	4.4 ± 0.3	4.6 ± 0.4	4.4 ± 0.4	4.6 ± 0.4	4.3 ± 0.4	4.7 ± 0.2	4.2 ± 0.2	0.09	0.94	0.31
fBCS	5.5[ab] ± 0.2	5.3[a] ± 0.2	5.8[bc] ± 0.2	6.2[cd] ± 0.2	6.3[d] ± 0.2	6.5 ± 0.2	5.1 ± 0.2	<0.01	<0.01	0.55
BCSG	0.88 ± 0.11	0.88 ± 0.13	0.88 ± 0.13	0.95 ± 0.13	0.81 ± 0.13	1.40 ± 0.08	0.36 ± 0.08	<0.01	0.96	0.98

[a-b]Within a variable, means differ ($P < 0.10$)

[nc]Without use of covariable

[1]iBW = initial BW, iSBW = initial shrunk BW, fSBW = final shrunk BW, SBG = shrunk body gain, rSBG = real shrunk body gain (discounting the gain related to pregnancy), iBCS = initial body condition score (1 to 9 scale), fBCS = final body condition score, BCSG = body condition score gain (points of BCS per each 100 days of experiment)

[2]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[3]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day). Initial variables were not tested.

Table 3. Chemical composition of cow's body

Item	Day of gestation					Feeding level[1]		P-value[2]		
	Empty (n = 12)	136 (n = 8)	189 (n = 8)	239 (n = 8)	269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
Total body										
N, kg	11.6[a] ± 0.2	11.1[a] ± 0.3	11.6[a] ± 0.3	12.3[b] ± 0.3	12.6[b] ± 0.3	12.7 ± 0.2	10.9 ± 0.2	<0.01	<0.01	0.61
Fat, kg	115 ± 4	108 ± 5	116 ± 5	125 ± 5	135 ± 5	137 ± 3	103 ± 3	<0.01	<0.01	0.03
High	135[c] ± 7	110[b] ± 7	133[c] ± 7	146[cd] ± 7	159[d] ± 7	-	-	-	-	-
Low	94[a] ± 6	106[ab] ± 7	99[ab] ± 7	104[ab] ± 7	110[b] ± 7	-	-	-	-	-
Ash, kg	19.1 ± 0.7	19.0 ± 0.9	19.7 ± 0.9	19.4 ± 0.9	19.8 ± 0.9	20.0 ± 0.5	18.8 ± 0.5	0.11	0.95	0.36
Water, kg	243[a] ± 4	242[a] ± 5	248[a] ± 5	274[b] ± 5	287[b] ± 5	275 ± 3	243 ± 3	<0.01	<0.01	0.38
Energy, Mcal	1,439 ± 42	1,351 ± 52	1,479 ± 52	1,574 ± 52	1,678 ± 52	1,695 ± 31	1,313 ± 31	<0.01	<0.01	0.03
High	1,656[c] ± 65	1,394[b] ± 73	1,667[c] ± 73	1,810[cd] ± 73	1,946[d] ± 73	-	-	-	-	-
Low	1,221[a] ± 55	1,307[ab] ± 73	1,291[ab] ± 73	1,338[ab] ± 73	1,410[b] ± 73	-	-	-	-	-
Non-pregnant body (EBW_{np})										
N, kg	11.6 ± 0.2	11.1 ± 0.3	11.4 ± 0.3	11.7 ± 0.3	11.8 ± 0.3	12.4 ± 0.2	10.6 ± 0.2	<0.01	0.27	0.62
Fat, kg	115 ± 4	108 ± 5	116 ± 5	124 ± 5	134 ± 5	136 ± 3	102 ± 3	<0.01	0.01	0.03
High	135[c] ± 7	110[b] ± 7	133[c] ± 7	145[cd] ± 7	158[d] ± 7	-	-	-	-	-
Low	94[a] ± 6	106[ab] ± 7	99[ab] ± 7	104[ab] ± 7	109[ab] ± 7	-	-	-	-	-
Ash, kg	19.1 ± 0.7	19.0 ± 0.9	19.4 ± 0.9	18.6 ± 0.9	18.6 ± 0.9	19.6 ± 0.5	18.3 ± 0.5	0.10	0.95	0.34
Water, kg	243 ± 4	234 ± 5	234 ± 5	243 ± 5	248 ± 5	266 ± 3	225 ± 3	<0.01	0.23	0.55
Energy, Mcal	1,439 ± 42	1,348 ± 52	1,472 ± 52	1,552 ± 52	1,646 ± 52	1682 ± 31	1301 ± 31	<0.01	<0.01	0.03
High	1,656[c] ± 65	1,392[b] ± 73	1,660[c] ± 73	1,788[cd] ± 73	1,912[d] ± 73	-	-	-	-	-
Low	1,221[a] ± 55	1,304[ab] ± 73	1,284[ab] ± 73	1,315[ab] ± 73	1,379[b] ± 73	-	-	-	-	-
Uterus										
N, kg	0.016[a] ± 0.02	0.071[b] ± 0.02	0.18[c] ± 0.02	0.56[d] ± 0.02	0.79[e] ± 0.02	0.33 ± 0.01	0.32 ± 0.01	0.43	<0.01	0.19

Fat, kg	0.017 ± 0.03	0.053 ± 0.04	0.18 ± 0.04	0.72 ± 0.04	1.01 ± 0.04	0.40 ± 0.02	0.39 ± 0.02	0.53	<0.01	0.07
High	0.023^{a} ± 0.05	0.047^{a} ± 0.05	0.18^{b} ± 0.05	0.66^{c} ± 0.05	1.11^{e} ± 0.05	-	-	-	-	-
Low	0.011^{a} ± 0.04	0.058^{a} ± 0.05	0.18^{b} ± 0.05	0.77^{c} ± 0.05	0.91^{d} ± 0.05	-	-	-	-	-
Ash, kg	0.006^{a} ± 0.03	0.085^{a} ± 0.04	0.22^{b} ± 0.04	0.77^{c} ± 0.04	1.17^{d} ± 0.04	0.44 ± 0.03	0.46 ± 0.03	0.70	<0.01	0.66
Water, kg	0.52 ± 0.98	8.09 ± 1.2	13.4 ± 1.2	30.5 ± 1.2	39.5 ± 1.2	18.8 ± 0.7	18.0 ± 0.7	0.41	<0.01	0.02
High	0.78^{a} ± 1.51	7.27^{b} ± 1.68	13.1^{c} ± 1.7	29.4^{d} ± 1.7	43.7^{f} ± 1.7	-	-	-	-	-
Low	0.25^{a} ± 1.28	8.90^{b} ± 1.68	13.8^{c} ± 1.7	31.6^{de} ± 1.7	35.4^{e} ± 1.7	-	-	-	-	-
Energy, Mcal	0.61^{a} ± 0.74	2.73^{b} ± 0.90	7.20^{c} ± 0.90	22.9^{d} ± 0.9	32.2^{e} ± 0.9	13.4 ± 0.6	12.8 ± 0.5	0.47	<0.01	0.35
				Total body						
N, g/kg	25.5 ± 0.3	25.1 ± 0.3	25.1 ± 0.3	24.6 ± 0.3	23.9 ± 0.3	24.7 ± 0.2	25.0 ± 0.2	0.27	0.01	0.04
High	24.8^{bc} ± 0.4	25.7^{cd} ± 0.5	24.9^{bc} ± 0.5	24.5^{b} ± 0.5	23.4^{a} ± 0.5	-	-	-	-	-
Low	26.1^{d} ± 0.4	24.4^{ab} ± 0.5	25.2^{bcd} ± 0.5	24.7^{bc} ± 0.5	24.5^{ab} ± 0.5	-	-	-	-	-
Fat, g/kg	247 ± 8	241 ± 10	249 ± 10	244 ± 10	253 ± 10	262 ± 6	231 ± 6	<0.01	0.92	0.17
Ash, g/kg	42.3 ± 1.7	42.7 ± 2.1	43.3 ± 2.1	38.7 ± 2.1	37.8 ± 2.1	39.1 ± 1.3	42.8 ± 1.3	0.05	0.23	0.20
Water, g/kg	536 ± 7	546 ± 8	538 ± 8	551 ± 8	547 ± 8	532 ± 5	555 ± 5	<0.01	0.58	0.44
Energy, Mcal/kg	3.11 ± 0.07	3.02 ± 0.08	3.18 ± 0.08	3.10 ± 0.08	3.16 ± 0.80	3.26 ± 0.05	2.97 ± 0.05	<0.01	0.68	0.15
				Non-pregnant body (EBW$_{np}$)						
N, g/kg	25.5 ± 0.3	25.4 ± 0.4	25.4 ± 0.4	25.3 ± 0.4	24.6 ± 0.4	25.0 ± 0.2	25.4 ± 0.2	0.18	0.38	0.07
High	24.8^{ab} ± 0.4	26.0^{c} ± 0.5	25.2^{abc} ± 0.5	25.1^{abc} ± 0.5	24.0^{a} ± 0.5	-	-	-	-	-
Low	26.1^{c} ± 0.4	24.7^{ab} ± 0.5	25.7^{bc} ± 0.5	25.4^{bc} ± 0.5	25.2^{abc} ± 0.5	-	-	-	-	-
Fat, g/kg	247 ± 8	245 ± 10	256 ± 10	261 ± 10	275 ± 10	272 ± 6	242 ± 6	<0.01	0.19	0.17
Ash, g/kg	42.3 ± 1.7	43.3 ± 2.2	44.2 ± 2.2	40.0 ± 2.2	39.1 ± 2.2	39.9 ± 1.4	43.7 ± 1.4	0.05	0.42	0.23
Water, g/kg	536 ± 7	537 ± 8	525 ± 8	527 ± 8	518 ± 8	517 ± 5	539 ± 5	<0.01	0.42	0.41
Energy, Mcal/kg	3.11^{ab} ± 0.07	3.07^{a} ± 0.08	3.27^{abc} ± 0.08	3.27^{bc} ± 0.08	3.40^{c} ± 0.08	3.37 ± 0.05	3.08 ± 0.05	<0.01	0.04	0.15
				Uterus						
N, g/kg	25.6^{d} ± 0.5	8.11^{a} ± 0.6	12.2^{b} ± 0.6	15.4^{c} ± 0.6	16.9^{c} ± 0.6	15.8 ± 0.4	15.6 ± 0.4	0.73	<0.01	0.58
Fat, g/kg	27.9^{d} ± 1.7	6.06^{a} ± 2.0	11.9^{b} ± 2.0	20.0^{c} ± 2.0	21.5^{c} ± 2.0	17.7 ± 1.2	17.2 ± 1.2	0.79	<0.01	0.92

Ash, g/kg	8.43 ± 0.49	9.89 ± 0.59	14.8 ± 0.6	21.5 ± 0.6	25.0 ± 0.6	15.6 ± 0.4	16.3 ± 0.4	0.18	<0.01	0.01
High	8.84[ab] ± 0.75	10.0[b] ± 0.8	15.4[c] ± 0.8	20.7[d] ± 0.8	23.0[e] ± 0.8	-	-	-	-	-
Low	8.03[a] ± 0.63	9.78[ab] ± 0.8	14.1[c] ± 0.8	22.3[de] ± 0.8	27.1[f] ± 0.8	-	-	-	-	-
Water, g/kg	799[a] ± 4	928[e] ± 5	889[d] ± 5	854[c] ± 5	839[b] ± 5	860 ± 3	863 ± 3	0.53	<0.01	0.40
Energy, Mcal/kg	0.98[d] ± 0.02	0.31[a] ± 0.02	0.48[b] ± 0.02	0.64[c] ± 0.02	0.69[c] ± 0.02	0.63 ± 0.01	0.62 ± 0.01	0.64	<0.01	0.48

[a-f]Within a variable, means differ ($P < 0.10$)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 4. Chemical composition of empty body gain of pregnant and non-pregnant Nellore cows

Item	Day of gestation					Feeding level[1]		P-value[2]		
	Empty (n = 12)	0 - 136 (n = 8)	0 - 189 (n = 8)	0 - 239 (n = 8)	0 - 269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
					Total gain					
N, kg	1.17 ± 0.18	0.77 ± 0.21	1.02 ± 0.21	1.23 ± 0.21	1.21 ± 0.21	2.01 ± 0.13	0.16 ± 0.13	<0.01	0.51	0.09
High	1.61[c] ± 0.27	1.74[c] ± 0.30	2.10[c] ± 0.30	2.30[c] ± 0.30	2.29[c] ± 0.30	-	-	-	-	-
Low	0.73[b] ± 0.23	-0.20[a] ± 0.30	-0.04[a] ± 0.30	0.17[ab] ± 0.30	0.14[ab] ± 0.30	-	-	-	-	-
Fat, kg	33.9 ± 5.4	25.3 ± 6.5	33.2 ± 6.5	41.2 ± 6.5	53.7 ± 6.5	51.8 ± 4.0	23.1 ± 3.9	<0.01	0.04	0.02
High	58.7[de] ± 8.3	20.6[ab] ± 9.2	48.2[cd] ± 9.2	58.4[de] ± 9.2	72.9[e] ± 9.2	-	-	-	-	-
Low	8.94[a] ± 7.0	29.9[bc] ± 9.2	18.2[ab] ± 9.2	24.1[ab] ± 9.2	34.5[bc] ± 9.2	-	-	-	-	-
Ash, kg	0.98 ± 0.44	0.92 ± 0.53	0.99 ± 0.53	0.88 ± 0.53	0.70 ± 0.53	1.88 ± 0.34	-0.091 ± 0.33	<0.01	0.99	0.02
High	0.64[abc] ± 0.67	2.68[d] ± 0.76	1.74[cd] ± 0.76	2.03[cd] ± 0.76	2.32[cd] ± 0.76	-	-	-	-	-
Low	1.31[bcd] ± 0.57	-0.85[a] ± 0.76	0.25[ab] ± 0.76	-0.26[ab] ± 0.76	-0.92[a] ± 0.76	-	-	-	-	-
Water, kg	18.9 ± 3.9	11.4 ± 4.7	13.1 ± 4.7	21.1 ± 4.7	23.6 ± 4.7	35.5 ± 2.9	-0.26 ± 2.82	<0.01	0.31	0.07
High	26.2[c] ± 5.9	29.8[c] ± 6.6	34.8[cd] ± 6.6	40.2[cd] ± 6.6	46.7[d] ± 6.6	-	-	-	-	-
Low	11.7[b] ± 5.0	-7.00[a] ± 6.62	-8.54[a] ± 6.62	2.00[ab] ± 6.6	0.50[ab] ± 6.6	-	-	-	-	-
Energy, Mcal	409 ± 50	319 ± 61	416 ± 61	483 ± 61	583 ± 61	602 ± 37	282 ± 37	<0.01	0.05	0.08
High	635[de] ± 77	331[ab] ± 86	566[cd] ± 86	675[de] ± 86	804[e] ± 86	-	-	-	-	-
Low	184[a] ± 65	307[ab] ± 86	266[ab] ± 86	291[ab] ± 86	362[bc] ± 86	-	-	-	-	-
					Daily gain					
N, g	10.3 ± 1.3	9.00 ± 1.52	6.82 ± 1.52	6.71 ± 1.52	5.51 ± 1.52	14.4 ± 0.9	0.89 ± 0.92	<0.01	0.13	0.01
High	14.3[c] ± 1.9	20.3[d] ± 2.1	14.3[c] ± 2.1	12.6[c] ± 2.1	10.7[bc] ± 2.1	-	-	-	-	-
Low	6.22[b] ± 1.62	-2.32[a] ± 2.15	-0.61[a] ± 2.15	0.80[a] ± 2.15	0.36[a] ± 2.15	-	-	-	-	-
Fat, g	274 ± 41	265 ± 50	222 ± 50	223 ± 50	245 ± 50	344 ± 31	148 ± 30	<0.01	0.90	0.01
High	492[e] ± 63	242[bcd] ± 71	326[d] ± 71	319[cd] ± 71	342[de] ± 71	-	-	-	-	-
Low	55[a] ± 53	288[bcd] ± 71	119[ab] ± 71	127[ab] ± 71	149[abc] ± 71	-	-	-	-	-
Ash, g	10.3 ± 4.1	11.6 ± 4.9	7.89 ± 4.91	4.79 ± 4.91	3.39 ± 4.91	14.8 ± 3.0	0.43 ± 2.97	<0.01	0.70	0.02

High	8.04^{bc} ± 6.21	32.1^{d} ± 6.9	11.7^{bc} ± 6.9	11.1^{bc} ± 6.9	10.9^{bc} ± 6.9	-	-	-	-	-
Low	12.6^{c} ± 5.2	-8.90^{a} ± 6.9	4.07^{abc} ± 6.9	-1.55^{abc} ± 6.9	-4.15^{ab} ± 6.9	-	-	-	-	-
Water, g	165 ± 30	138 ± 36	83 ± 36	115 ± 36	107 ± 36	251 ± 22	-7.75 ± 21.76	<0.01	0.47	0.04
High	231^{c} ± 46	350^{d} ± 51	236^{cd} ± 51	220^{c} ± 51	217^{c} ± 51	-	-	-	-	-
Low	99^{b} ± 38	-74.7^{a} ± 51	-70.7^{a} ± 51	9.91^{ab} ± 51	-2.09^{ab} ± 51	-	-	-	-	-
Energy, Mcal	3.48 ± 0.35	3.42 ± 0.43	2.89 ± 0.43	2.61 ± 0.43	2.67 ± 0.43	4.13 ± 0.27	1.89 ± 0.26	<0.01	0.40	0.11
High	5.46^{d} ± 0.54	3.89^{c} ± 0.61	3.83^{c} ± 0.61	3.69^{c} ± 0.61	3.78^{c} ± 0.61	-	-	-	-	-
Low	1.50^{a} ± 0.46	2.94^{bc} ± 0.61	1.94^{ab} ± 0.61	1.53^{ab} ± 0.61	1.55^{ab} ± 0.61	-	-	-	-	-

[a-e]Within a variable, means differ ($P < 0.10$)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 5. Body parts, organs and viscera and gastrointestinal parts of pregnant and non-pregnant cows in function of days of gestation and feeding level, presented as fresh weights

Item[1]	Day of gestation					Feeding level[2]		P-value[3]		
	Empty (n = 12)	136 (n = 8)	189 (n = 8)	239 (n = 8)	269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
Cows body parts, kg										
Carcass	303^{ab} ± 6	290^{a} ± 7	297^{a} ± 7	316^{bc} ± 7	322^{c} ± 7	329 ± 4	282 ± 4	<0.01	0.01	0.12
Non-carcass	148 ± 3	142 ± 3	147 ± 3	145 ± 3	152 ± 3	161 ± 2	132 ± 2	<0.01	0.20	0.63
Head	13.5 ± 0.3	13.3 ± 0.3	13.5 ± 0.3	13.4 ± 0.3	13.8 ± 0.3	13.8 ± 0.2	13.2 ± 0.2	0.07	0.87	0.59
Feet	8.57 ± 0.24	8.73 ± 0.29	8.68 ± 0.29	9.11 ± 0.29	9.13 ± 0.29	9.31 ± 0.18	8.37 ± 0.18	<0.01	0.49	0.70
Hide	40.3 ± 1.0	36.5 ± 1.3	39.9 ± 1.3	38.3 ± 1.3	40.1 ± 1.3	40.9 ± 0.8	37.1 ± 0.8	<0.01	0.16	0.77
Blood	16.7^{abc} ± 0.5	17.4^{bc} ± 0.5	15.6^{a} ± 0.5	16.2^{ab} ± 0.5	17.7^{c} ± 0.5	18.0 ± 0.3	15.4 ± 0.3	<0.01	0.06	0.94
Organs and viscera	69.1 ± 2.0	65.6 ± 2.4	68.9 ± 2.4	68.3 ± 2.4	71.4 ± 2.4	79.2 ± 1.5	58.1 ± 1.4	<0.01	0.54	0.46
Uterus	0.64 ± 1.14	8.71 ± 1.38	15.1 ± 1.4	35.8 ± 1.4	47.0 ± 1.4	21.9 ± 0.9	21.0 ± 0.8	0.41	<0.01	0.03
High	0.95^{a} ± 1.75	7.87^{b} ± 1.94	14.8^{c} ± 1.9	34.5^{d} ± 1.9	51.7^{f} ± 1.9	-	-	-	-	-
Low	0.34^{a} ± 1.47	9.54^{b} ± 1.94	15.5^{c} ± 1.9	37.1^{de} ± 1.9	42.4^{e} ± 1.9	-	-	-	-	-
Udder	4.57 ± 0.30	4.28 ± 0.37	4.16 ± 0.37	4.34 ± 0.37	6.02 ± 0.37	5.28 ± 0.22	4.07 ± 0.22	<0.01	<0.01	0.07
High	5.01^{bc} ± 0.47	4.66^{abc} ± 0.52	4.10^{abc} ± 0.52	5.20^{c} ± 0.52	7.45^{d} ± 0.52	-	-	-	-	-
Low	4.14^{abc} ± 0.40	3.90^{ab} ± 0.52	4.22^{abc} ± 0.52	3.49^{a} ± 0.52	4.59^{abc} ± 0.52	-	-	-	-	-
Cows organs and viscera, kg										
Heart	1.66 ± 0.05	1.52 ± 0.07	1.56 ± 0.07	1.65 ± 0.07	1.60 ± 0.07	1.63 ± 0.04	1.57 ± 0.04	0.28	0.47	0.76
Liver	5.10^{b} ± 0.13	4.68^{a} ± 0.15	4.83^{ab} ± 0.15	4.74^{a} ± 0.15	4.47^{a} ± 0.15	5.54 ± 0.09	3.99 ± 0.09	<0.01	0.03	0.39
Kidneys	0.78 ± 0.03	0.73 ± 0.03	0.77 ± 0.03	0.72 ± 0.03	0.71 ± 0.03	0.81 ± 0.02	0.67 ± 0.02	<0.01	0.45	0.56
Lungs	2.28 ± 0.09	2.11 ± 0.11	2.36 ± 0.11	2.33 ± 0.11	2.10 ± 0.11	2.37 ± 0.07	2.10 ± 0.07	<0.01	0.28	0.48

Diaphragm	2.20 ± 0.10	2.35 ± 0.13	2.26 ± 0.13	2.47 ± 0.13	2.57 ± 0.13	2.67 ± 0.08	2.07 ± 0.08	<0.01	0.21	0.39
Spleen	0.96 ± 0.05	0.85 ± 0.06	0.89 ± 0.06	0.95 ± 0.06	0.89 ± 0.06	1.02 ± 0.04	0.80 ± 0.04	<0.01	0.66	0.59
Visceral fat	9.46 ± 0.62	8.20 ± 0.75	9.00 ± 0.75	9.39 ± 0.75	10.7 ± 0.75	11.6 ± 0.46	7.12 ± 0.45	<0.01	0.23	0.01
High	11.8[de] ± 0.95	8.74[bc] ± 1.06	11.9[de] ± 1.09	13.4[e] ± 1.06	11.9[de] ± 1.06	-	-	-	-	-
Low	7.09[ab] ± 0.80	7.65[abc] ± 1.06	6.10[a] ± 1.06	5.19[a] ± 1.06	9.58[cd] ± 1.06	-	-	-	-	-
Mesentery	22.7 ± 1.4	21.5 ± 1.8	23.1 ± 1.8	22.2 ± 1.8	25.1 ± 1.8	28.1 ± 1.1	17.7 ± 1.1	<0.01	0.65	0.88
Gastrointestinal tract	17.0 ± 0.4	17.1 ± 0.5	17.1 ± 0.5	16.7 ± 0.5	15.8 ± 0.5	18.1 ± 0.3	15.4 ± 0.3	<0.01	0.29	0.67
				Gastrointestinal tract dissection, kg						
Rumen-reticulum	7.41 ± 0.21	7.43 ± 0.26	7.35 ± 0.26	7.08 ± 0.26	6.95 ± 0.26	7.86 ± 0.16	6.63 ± 0.16	<0.01	0.56	0.73
Omasum	2.19 ± 0.12	2.37 ± 0.14	2.35 ± 0.14	2.17 ± 0.14	1.97 ± 0.14	2.47 ± 0.09	1.95 ± 0.09	<0.01	0.27	0.71
Abomasum	1.23 ± 0.05	1.11 ± 0.06	1.10 ± 0.06	1.14 ± 0.06	1.14 ± 0.06	1.24 ± 0.04	1.05 ± 0.04	<0.01	0.47	0.51
Small intestine	3.71 ± 0.16	3.78 ± 0.20	3.97 ± 0.20	3.98 ± 0.20	3.50 ± 0.20	4.01 ± 0.12	3.57 ± 0.12	0.01	0.39	0.62
Large intestine	2.51 ± 0.11	2.43 ± 0.13	2.38 ± 0.13	2.34 ± 0.13	2.25 ± 0.13	2.50 ± 0.08	2.26 ± 0.08	0.03	0.59	0.84
Total stomach	10.8 ± 0.3	10.9 ± 0.3	10.8 ± 0.3	10.4 ± 0.3	10.1 ± 0.3	11.6 ± 0.20	9.62 ± 0.20	<0.01	0.28	0.52
Total intestine	6.22 ± 0.24	6.21 ± 0.29	6.34 ± 0.29	6.32 ± 0.29	5.75 ± 0.29	6.51 ± 0.18	5.82 ± 0.17	<0.01	0.59	0.79

[a-e]Within a variable, means differ ($P < 0.10$)

[1]Non-carcass = head + feet + hide + blood, total stomach = rumen-reticulum + omasum + abomasum and total intestine = small intestine + large intestine.

[2]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[3]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 6. Body parts, organs and viscera and gastrointestinal parts of pregnant and non-pregnant cows in function of days of gestation and feeding level, presented as proportion of non-pregnant empty BW (EBW_{np})

Item[1]	Day of gestation					Feeding level[2]		P-value[3]		
	Empty (n = 12)	136 (n = 8)	189 (n = 8)	239 (n = 8)	269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
				Cows body parts, g/kg of EBW_{np}						
Carcass	663 ± 5	664 ± 6	662 ± 6	677 ± 6	670 ± 6	663 ± 4	671 ± 4	0.11	0.35	0.61
Non-carcass	326 ± 5	325 ± 6	327 ± 6	313 ± 6	317 ± 6	325 ± 4	318 ± 4	0.17	0.33	0.61
Head	29.8 ± 0.6	30.7 ± 0.8	30.4 ± 0.8	29.4 ± 0.8	29.0 ± 0.8	27.9 ± 0.5	31.9 ± 0.5	<0.01	0.49	0.79
Feet	19.0 ± 0.6	20.2 ± 0.7	19.6 ± 0.7	19.8 ± 0.7	19.2 ± 0.7	18.9 ± 0.4	20.2 ± 0.4	0.03	0.73	0.27
Hide	89.8 ± 2.4	83.9 ± 2.9	89.6 ± 2.9	82.8 ± 2.9	83.7 ± 2.9	82.8 ± 1.8	89.1 ± 1.8	0.01	0.21	0.55
Blood	37.0^{a} ± 1.1	40.1^{b} ± 1.4	34.9^{a} ± 1.4	35.3^{a} ± 1.4	37.0^{ab} ± 1.4	36.4 ± 0.8	37.4 ± 0.8	0.40	0.07	0.87
Organs and viscera	150 ± 4	150 ± 4	153 ± 4	145 ± 4	148 ± 4	159 ± 3	139 ± 3	<0.01	0.79	0.63
Udder	10.0^{a} ± 0.60	9.81^{a} ± 0.72	9.39^{a} ± 0.72	9.26^{a} ± 0.72	12.4^{b} ± 0.72	10.6 ± 0.45	9.71 ± 0.44	0.14	0.03	0.14
				Cows organs and viscera, *g/kg of EBW_{np}*						
Heart	3.65 ± 0.13	3.55 ± 0.16	3.50 ± 0.16	3.61 ± 0.16	3.38 ± 0.16	3.30 ± 0.10	3.78 ± 0.09	<0.01	0.72	0.46
Liver	11.2^{c} ± 0.2	10.8^{bc} ± 0.3	10.7^{bc} ± 0.3	10.1^{b} ± 0.3	9.18^{a} ± 0.3	11.2 ± 0.17	9.59 ± 0.17	<0.01	<0.01	0.25
Kidneys	1.72 ± 0.06	1.66 ± 0.08	1.73 ± 0.08	1.57 ± 0.08	1.48 ± 0.08	1.64 ± 0.04	1.63 ± 0.04	0.85	0.10	0.44
Lungs	4.99 ± 0.22	4.88 ± 0.27	5.34 ± 0.27	5.05 ± 0.27	4.40 ± 0.27	4.80 ± 0.16	5.06 ± 0.16	0.25	0.17	0.45
Diaphragm	4.78 ± 0.20	5.41 ± 0.25	5.06 ± 0.25	5.28 ± 0.25	5.26 ± 0.25	5.36 ± 0.15	4.96 ± 0.16	0.07	0.35	0.41
Spleen	2.14 ± 0.11	1.95 ± 0.14	1.98 ± 0.14	2.08 ± 0.14	1.81 ± 0.14	2.05 ± 0.09	1.93 ± 0.08	0.30	0.43	0.61
Visceral fat	20.1 ± 1.4	18.4 ± 1.6	19.6 ± 1.6	19.2 ± 1.6	22.2 ± 1.6	23.2 ± 1.0	16.7 ± 1.0	<0.01	0.58	0.02
High	23.9^{de} ± 2.1	19.1^{bcd} ± 2.3	24.5^{de} ± 2.3	26.2^{e} ± 2.3	22.1^{cde} ± 2.3	-	-	-	-	-
Low	16.3^{sb} ± 1.7	17.8^{bc} ± 2.3	14.8^{ab} ± 2.3	12.2^{a} ± 2.3	22.2^{cde} ± 2.3	-	-	-	-	-

Mesentery	48.6 ± 2.8	48.6 ± 3.4	50.5 ± 3.4	46.6 ± 3.4	51.1 ± 3.4	55.9 ± 2.1	42.3 ± 2.1	<0.01	0.89	0.94
Gastrointestinal tract	37.9[b] ± 1.1	39.4[b] ± 1.4	38.7[b] ± 1.4	36.5[b] ± 1.4	32.8[a] ± 1.4	36.8 ± 0.8	37.3 ± 0.8	0.65	0.01	0.32
Gastrointestinal tract dissection, *g/kg of* EBW_{np}										
Rumen-reticulum	16.3[bc] ± 0.5	17.1[c] ± 0.6	16.5[bc] ± 0.6	15.3[ab] ± 0.6	14.4[a] ± 0.6	15.9 ± 0.3	15.9 ± 0.3	0.94	0.01	0.21
Omasum	4.83[b] ± 0.27	5.40[b] ± 0.33	5.29[b] ± 0.33	4.77[ab] ± 0.33	4.07[a] ± 0.33	5.02 ± 0.20	4.73 ± 0.20	0.29	0.05	0.55
Abomasum	2.76 ± 0.13	2.53 ± 0.16	2.46 ± 0.16	2.49 ± 0.16	2.36 ± 0.16	2.51 ± 0.10	2.52 ± 0.10	0.93	0.31	0.61
Small intestine	8.27 ± 0.43	8.81 ± 0.53	9.04 ± 0.53	8.79 ± 0.53	7.26 ± 0.53	8.21 ± 0.32	8.66 ± 0.32	0.32	0.14	0.60
Large intestine	5.67 ± 0.29	5.59 ± 0.36	5.40 ± 0.36	5.14 ± 0.36	4.71 ± 0.36	5.14 ± 0.22	5.47 ± 0.21	0.29	0.28	0.73
Total stomach	23.9[bc] ± 0.6	25.0[c] ± 0.7	24.3[bc] ± 0.7	22.5[ab] ± 0.7	20.8[a] ± 0.7	23.4 ± 0.4	23.2 ± 0.4	0.68	<0.01	0.11
Total intestine	13.9 ± 0.6	14.4 ± 0.8	14.4 ± 0.8	13.9 ± 0.8	12.0 ± 0.8	13.3 ± 0.5	14.1 ± 0.5	0.26	0.19	0.70

[a-d]Within a variable, means differ (P < 0.10)

[1]Non-carcass = head + feet + hide + blood, total stomach = rumen-reticulum + omasum + abomasum and total intestine = small intestine + large intestine.

[2]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[3]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 7. Statistics describing relationships between gravid uterus components and days of gestation (t)

Function	Parameter			Residual		F^{a}	*P*-value
	A ± SE	B ± SE	C ± SE	Sum of squares	df		
Gravid uterus $= Ae^{(B - Ct)t}$							
HIGH-fed cows	0.3633 ± 0.76	0.02325 ± 0.02	0.00001798 ± 0.00004	327	13	2.2	0.10
LOW-fed cows	0.0636 ± 0.14	0.04376 ± 0.02	0.00007287 ± 0.00004	397	13		
Pooled	0.1475 ± 0.24	0.03387 ± 0.01	0.00004639 ± 0.00003	911	29		
Fetus $= Ae^{(B - Ct)t}$							
HIGH-fed cows	0.0003202 ± 0.0009	0.07573 ± 0.02	0.0001234 ± 0.0005	56.2	13	3.2	0.04
LOW-fed cows	0.0000225 ± 0.0001	0.10225 ± 0.03	0.0001880 ± 0.0006	90.2	13		
Pooled	0.0000720 ± 0.0002	0.09040 ± 0.02	0.0001587 ± 0.0005	200.3	29		
Uterus $= Ae^{(B - Ct)t}$							
HIGH-fed cows	0.2136 ± 0.46	0.01375 ± 0.02	0.00000028 ± 0.00004	14.4	13	1.5	0.24
LOW-fed cows	0.0149 ± 0.04	0.04454 ± 0.02	0.00007979 ± 0.00005	23.7	13		
Pooled	0.0585 ± 0.10	0.02910 ± 0.01	0.00004043 ± 0.00004	44.5	29		
Placenta $= Ae^{(B - Ct)t}$							
HIGH-fed cows	0.05511 ± 0.08	0.02123 ± 0.01	0.00003229 ± 0.00003	0.67	13	4.1	0.01
LOW-fed cows	0.01729 ± 0.02	0.02947 ± 0.01	0.00004993 ± 0.00003	0.29	13		
Pooled	0.03279 ± 0.04	0.02493 ± 0.01	0.00004032 ± 0.00003	1.41	29		
Fetal fluids $= Ae^{(Bt)}$							
HIGH-fed cows	0.673 ± 0.3	0.01033 ± 0.002	-	52.2	14	2.7	0.08

LOW-fed cows	2.406 ± 0.7	0.00482 ± 0.001	-	37.6	14
Pooled	1.389 ± 0.4	0.00723 ± 0.001	-	106.8	30

[a]F-ratio test the hypothesis that the feeding level functions are equivalent. The *P*-value provide evidence that a common function is inappropriate and that relationships between gravid uterus components differ between feeding levels.

Table 8. Udder chemical composition in pregnant and non-pregnant cows as function of days of gestation and feeding level

Item[1]	Day of gestation					Feeding level[2]		P-value[3]		
	Empty (n = 12)	136 (n = 8)	189 (n = 8)	239 (n = 8)	269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
Fresh weight[4], kg	4.57 ± 0.30	4.28 ± 0.37	4.16 ± 0.37	4.34 ± 0.37	6.02 ± 0.37	5.28 ± 0.22	4.07 ± 0.22	<0.01	<0.01	0.07
High	5.01^{bc} ± 0.47	4.66^{abc} ± 0.52	4.10^{abc} ± 0.52	5.20^{c} ± 0.52	7.45^{d} ± 0.52	-	-	-	-	-
Low	4.14^{abc} ± 0.40	3.90^{ab} ± 0.52	4.22^{abc} ± 0.52	3.49^{a} ± 0.52	4.59^{abc} ± 0.52	-	-	-	-	-
Dry matter, kg	4.09 ± 0.30	3.72 ± 0.36	3.32 ± 0.36	3.38 ± 0.36	4.07 ± 0.36	4.29 ± 0.22	3.14 ± 0.22	<0.01	0.35	0.03
High	4.43^{de} ± 0.46	4.00^{cd} ± 0.51	3.19^{abc} ± 0.51	4.40^{cde} ± 0.51	5.46^{e} ± 0.51	-	-	-	-	-
Low	3.76^{bcd} ± 0.38	3.45^{abcd} ± 0.51	3.45^{abcd} ± 0.51	2.37^{a} ± 0.51	2.69^{ab} ± 0.51	-	-	-	-	-
N, kg	0.078 ± 0.006	0.085 ± 0.007	0.082 ± 0.007	0.090 ± 0.007	0.135 ± 0.007	0.103 ± 0.004	0.085 ± 0.004	<0.01	<0.01	0.03
High	0.078^{ab} ± 0.009	0.096^{abc} ± 0.01	0.080^{ab} ± 0.01	0.098^{bc} ± 0.01	0.163^{d} ± 0.01	-	-	-	-	-
Low	0.078^{ab} ± 0.008	0.073^{a} ± 0.01	0.083^{abc} ± 0.01	0.082^{ab} ± 0.01	0.108^{c} ± 0.01	-	-	-	-	-
Fat, kg	3.38 ± 0.26	3.00 ± 0.31	2.65 ± 0.31	2.56 ± 0.31	2.93 ± 0.31	3.37 ± 0.19	2.43 ± 0.19	<0.01	0.27	0.03
High	3.72^{de} ± 0.39	3.19^{cde} ± 0.44	2.47^{abc} ± 0.44	3.42^{cde} ± 0.44	4.07^{e} ± 0.44	-	-	-	-	-
Low	3.03^{cd} ± 0.33	2.81^{bcd} ± 0.44	2.82^{bcd} ± 0.44	1.70^{a} ± 0.44	1.81^{ab} ± 0.44	-	-	-	-	-
Ash, kg	0.022^{a} ± 0.003	0.023^{ab} ± 0.003	0.023^{ab} ± 0.003	0.029^{b} ± 0.003	0.042^{c} ± 0.003	0.031 ± 0.002	0.025 ± 0.002	0.02	<0.01	0.23
Water, kg	0.46^{a} ± 0.10	0.66^{ab} ± 0.12	0.73^{bc} ± 0.12	1.02^{c} ± 0.12	1.86^{d} ± 0.12	0.98 ± 0.07	0.92 ± 0.07	0.57	<0.01	0.52
Energy, Mcal	34.6 ± 2.5	30.5 ± 3.1	28.0 ± 3.1	27.4 ± 3.1	32.2 ± 3.1	35.2 ± 1.9	25.9 ± 1.8	<0.01	0.35	0.02
High	37.7^{de} ± 3.9	32.3^{cd} ± 4.3	26.2^{abc} ± 4.3	35.8^{cde} ± 4.3	43.9^{e} ± 4.3	-	-	-	-	-
Low	31.4^{cd} ± 3.3	28.7^{bcd} ± 4.3	29.8^{bcd} ± 4.3	19.0^{a} ± 4.3	20.5^{ab} ± 4.3	-	-	-	-	-
Dry matter, g/kg	895 ± 17	841 ± 21	813 ± 21	748 ± 21	677 ± 21	820 ± 13	769 ± 13	<0.01	<0.01	0.02
High	907^{f} ± 26	837de ± 29	802^{cd} ± 29	821de ± 29	736^{bc} ± 29	-	-	-	-	-
Low	883^{ef} ± 22	845^{def} ± 29	823de ± 29	675^{ab} ± 29	618^{a} ± 29	-	-	-	-	-
N, g/kg	17.5^{a} ± 0.9	19.5^{ab} ± 1.2	20.2^{b} ± 1.2	21.1^{bc} ± 1.2	23.4^{c} ± 1.2	19.6 ± 0.74	21.1 ± 0.72	0.15	0.01	0.44
Fat, g/kg	736 ± 21	676 ± 26	644 ± 26	559 ± 26	485 ± 26	647 ± 16	593 ± 16	0.02	<0.01	0.03

High	762^{f} ± 32	664^{cde} ± 36	621^{cd} ± 36	634^{cde} ± 36	554^{bc} ± 36	-	-	-	-	-
Low	709^{ef} ± 27	687^{def} ± 36	668^{def} ± 36	484^{ab} ± 36	418^{a} ± 36	-	-	-	-	-
Ash, g/kg	4.76^{a} ± 0.34	5.28^{ab} ± 0.41	5.66^{bc} ± 0.41	6.57^{cd} ± 0.41	7.12^{d} ± 0.41	5.69 ± 0.25	6.06 ± 0.25	0.29	<0.01	0.77
Water, g/kg	105 ± 17	159 ± 21	187 ± 21	252 ± 21	323 ± 21	180 ± 13	231 ± 13	<0.01	<0.01	0.02
High	93^{a} ± 26	163^{bc} ± 29	198^{cd} ± 29	179^{bc} ± 29	264de ± 29	-	-	-	-	-
Low	117^{ab} ± 22	155^{abc} ± 29	177^{bc} ± 29	325^{ef} ± 29	382^{f} ± 29	-	-	-	-	-
Energy, Mcal/kg	7.55 ± 0.20	6.90 ± 0.24	6.79 ± 0.24	6.04 ± 0.24	5.35 ± 0.24	6.74 ± 0.15	6.31 ± 0.14	0.04	<0.01	0.03
High	7.75^{f} ± 0.30	6.76^{cde} ± 0.34	6.55^{cd} ± 0.34	6.66^{cde} ± 0.34	5.98^{bc} ± 0.34	-	-	-	-	-
Low	7.35^{ef} ± 0.26	7.03^{def} ± 0.34	7.02^{def} ± 0.34	5.41^{ab} ± 0.34	4.72^{a} ± 0.34	-	-	-	-	-

[a-f]Within a variable, means differ ($P < 0.10$)

[1]FW = udder fresh weight

[2]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[3]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

[4]The values of fresh weight and its interaction are the same presented in Table 5.

Table 9. Development of gravid uterus components in function of days of gestation and feeding level in pregnant Nellore cows

Item	Day of gestation				SEM	Feeding level[1]		SEM	P-value[2]		
	136	189	239	269		High	Low		FL	Day	FL × day
Fetus, kg	1.50	6.33	19.9	27.1	0.93	14.3	13.0	0.66	0.17	<0.01	0.01
High	1.45^{a}	6.47^{b}	19.8^{c}	29.7^{d}	1.12	-	-	-	-	-	-
Low	1.55^{a}	6.19^{b}	20.0^{c}	24.4^{c}	1.12	-	-	-	-	-	-
Fetus, g/kg of GU	171^{a}	431^{b}	555^{c}	579^{c}	14	444	423	9	0.15	<0.01	0.73
High	180	447	573	577	19	-	-	-	-	-	-
Low	162	414	539	581	19	-	-	-	-	-	-
Uterus, kg	2.35^{a}	3.36^{a}	6.96^{b}	8.36^{c}	0.40	5.16	5.36	0.28	0.61	<0.01	0.12
High	2.11	3.18	6.28	9.06	0.56	-	-	-	-	-	-
Low	2.61	3.54	7.65	7.65	0.56	-	-	-	-	-	-
Uterus, g/kg of GU	263^{a}	227^{b}	193^{c}	178^{c}	7	208	221	5	0.07	<0.01	0.77
High	260	219	181	174	10	-	-	-	-	-	-
Low	265	234	204	182	10	-	-	-	-	-	-
Placenta, kg	0.47^{a}	0.84^{b}	1.27^{c}	1.45^{c}	0.06	1.12	0.90	0.04	<0.01	<0.01	0.18
High	0.51	1.03	1.31	1.65	0.09	-	-	-	-	-	-
Low	0.44	0.66	1.22	1.26	0.09	-	-	-	-	-	-
Placenta, g/kg of GU	54.4	57.1	35.9	31.0	2.4	50.5	38.7	1.7	<0.01	<0.01	0.01
High	62.2^{c}	69.3^{c}	39.1^{ab}	31.4^{a}	3.5	-	-	-	-	-	-
Low	46.7^{b}	45.0^{ab}	32.7^{ab}	30.5^{a}	3.5	-	-	-	-	-	-
Fetal fluids, kg	4.61^{a}	4.35^{a}	7.79^{b}	9.98^{c}	0.58	6.61	6.75	0.41	0.81	<0.01	0.06
High	4.00^{ab}	3.81^{a}	7.31^{bcd}	11.3^{e}	0.82	-	-	-	-	-	-
Low	5.22^{abcd}	4.91^{abc}	8.26^{cde}	8.62^{de}	0.82	-	-	-	-	-	-
Fetal fluids, g/kg of GU	512^{a}	285^{b}	216^{c}	212^{c}	14	296	316	10	0.17	<0.01	0.55
High	497	262	208	217	20	-	-	-	-	-	-
Low	526	308	223	207	20	-	-	-	-	-	-

[a-e]Within a variable, means differ (P < 0.10)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 10. Development of fetus body compounds and organs and viscera in function of day of gestation and feeding level in pregnant Nellore cows, presented as fresh weight

Item	Day of gestation				SEM	Feeding level[1]		SEM	P-value[2]		
	136	189	239	269		High	Low		FL	Day	FL × day
					Fetus body compounds, g						
Head	242	803	1,891	2,377	49	1,362	1,294	35	0.18	<0.01	0.05
High	240[a]	789[b]	1,880[c]	2,537[e]	69	-	-	-	-	-	-
Low	244[a]	816[b]	1,902[c]	2,217[d]	69	-	-	-	-	-	-
Feet	61.0[a]	348[a]	1,519[b]	1,846[c]	74	962	925	52	0.62	<0.01	0.85
High	60.2	352	1516	1920	105	-	-	-	-	-	-
Low	61.8	344	1522	1771	105	-	-	-	-	-	-
Carcass	829	3,659	12,560	17,048	478	9,052	7,996	338	0.03	<0.01	<0.01
High	805[a]	3,811[b]	12,380[c]	19,211[d]	676	-	-	-	-	-	-
Low	854[a]	3,507[b]	12,739[c]	14,884[c]	676	-	-	-	-	-	-
Organs and viscera	174[a]	646[b]	1,887[c]	2,623[d]	82	1,378	1,286	58	0.27	<0.01	0.23
High	169	634	1889	2821	117	-	-	-	-	-	-
Low	179	658	1884	2424	117	-	-	-	-	-	-
					Fetus organs and viscera, g						
Heart	11.7[a]	37.2[a]	129[b]	166[c]	6.6	89.4	82.6	4.7	0.31	<0.01	0.57
High	11.5	38.0	131	177	9.4	-	-	-	-	-	-
Low	12.0	36.4	128	154	9.4	-	-	-	-	-	-
Lungs	38.6[a]	137[b]	365[c]	567[d]	18.1	290	264	13	0.16	<0.01	0.68
High	37.9	145	382	596	25.7	-	-	-	-	-	-
Low	39.4	130	347	538	25.7	-	-	-	-	-	-
Kidneys	18.2[a]	63.7[b]	151[c]	167[c]	9.2	107	93.1	6.5	0.14	<0.01	0.13
High	18.4	57.5	160	191	13.0	-	-	-	-	-	-
Low	18.0	69.8	142	143	13.0	-	-	-	-	-	-
Liver	50.1[a]	170[b]	421[c]	544[d]	26	310	283	19	0.30	<0.01	0.21

High	48.9	163	421	608	37	-	-	-	-	-	-
Low	51.4	178	421	481	37	-	-	-	-	-	-
Spleen	3.10[a]	14.5[b]	35.6[c]	46.0[d]	2.1	26.5	23.0	1.5	0.10	<0.01	0.52
High	2.88	15.6	37.7	50.0	2.9	-	-	-	-	-	-
Low	3.31	13.4	33.5	42.0	2.9	-	-	-	-	-	-
Pancreas	0.85[a]	2.71[b]	7.68[c]	10.6[d]	0.47	5.70	5.22	0.33	0.32	<0.01	0.32
High	0.89	2.75	7.56	11.6	0.67	-	-	-	-	-	-
Low	0.82	2.67	7.81	9.57	0.67	-	-	-	-	-	-
Gastrointestinal tract	40.5[a]	159[b]	510[c]	650[d]	20	333	346	14	0.51	<0.01	0.53

[a-e]Within a variable, means differ (P < 0.10)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 11. Development of fetus body compounds and organs and viscera in function of day of gestation and feeding level in pregnant Nellore cows, presented as proportion of fetus BW

Item	Day of gestation				SEM	Feeding level[1]		SEM	P-value[2]		
	136	189	239	269		High	Low		FL	Day	FL × day
				Fetus body compounds,g/kg of fetus BW							
Head	163[c]	129[b]	95.9[a]	88.5[a]	3.7	118	120	3	0.54	<0.01	0.29
High	167	123	95.3	86.0	5.3	-	-	-	-	-	-
Low	159	135	96.5	91.0	5.3	-	-	-	-	-	-
Feet	40.3[a]	54.7[b]	75.9[d]	68.5[c]	1.8	59.2	60.5	1.3	0.44	<0.01	0.24
High	40.7	54.6	76.7	64.6	2.6	-	-	-	-	-	-
Low	39.8	54.7	75.2	72.3	2.6	-	-	-	-	-	-
Carcass	551[a]	576[a]	630[b]	630[b]	9	603	590	7	0.20	<0.01	0.28
High	551	586	625	648	13	-	-	-	-	-	-
Low	551	564	635	612	13	-	-	-	-	-	-
Organs and viscera	116[b]	102[b]	95.2[a]	96.9[ab]	2.4	101	104	2	0.22	<0.01	0.52
High	116	98.4	95.5	94.6	3.4	-	-	-	-	-	-
Low	116	106	95.0	99.4	3.4	-	-	-	-	-	-
				Fetus organs and viscera, g/kg of fetus BW							
Heart	7.86[b]	5.86[a]	6.47[a]	6.15[a]	0.27	6.57	6.61	0.19	0.89	<0.01	0.87
High	7.88	5.87	6.57	5.95	0.39	-	-	-	-	-	-
Low	7.84	5.85	6.37	6.36	0.39	-	-	-	-	-	-
Lungs	25.6[b]	21.5[a]	18.6[a]	21.1[a]	1.1	21.9	21.5	0.8	0.75	<0.01	0.62
High	25.8	22.3	19.4	20.1	1.6	-	-	-	-	-	-
Low	25.5	20.7	17.9	22.2	1.6	-	-	-	-	-	-
Kidneys	12.1[c]	10.1[b]	7.70[a]	6.06[a]	0.53	8.98	9.02	0.37	0.95	<0.01	0.11
High	12.5	8.93	8.11	6.35	0.75	-	-	-	-	-	-

Low	11.7	11.3	7.30	5.78	0.75	-	-	-	-	-	-
Liver	33.5[c]	27.3[b]	21.0[a]	20.1[a]	1.0	25.2	25.8	0.7	0.57	<0.01	0.33
High	33.8	25.4	31.1	20.4	1.4	-	-	-	-	-	-
Low	33.2	29.2	21.0	19.7	1.4	-	-	-	-	-	-
Spleen	2.08[ab]	2.27[b]	1.79[a]	1.70[a]	0.13	2.00	1.92	0.09	0.52	0.02	0.56
High	2.00	2.42	1.90	1.68	0.19	-	-	-	-	-	-
Low	2.16	2.11	1.68	1.72	0.19	-	-	-	-	-	-
Pancreas	0.60[b]	0.43[a]	0.39[a]	0.39[a]	0.04	0.46	0.44	0.03	0.57	<0.01	0.57
High	0.66	0.42	0.38	0.39	0.06	-	-	-	-	-	-
Low	0.54	0.44	0.40	0.39	0.06	-	-	-	-	-	-
Gastrointestinal tract	27.3	25.3	25.9	25.8	1.1	25.2	27.0	0.75	0.11	0.58	0.66
High	26.9	23.8	25.9	24.3	1.6	-	-	-	-	-	-
Low	27.8	26.9	25.9	27.3	1.6	-	-	-	-	-	-

[a-d]Within a variable, means differ ($P < 0.10$)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 12. Equations to explain fetal organs development in function of days of gestation in pregnant Nellore cows

Organ	Function	D50[1]	Proportion of BW, g/kg		
			145 days of gestation	At parturition (predicted)	Adult cow[2]
Heart	$f(t)$, g = $0.0000646e^{(0.1074 - 0.000195t)t}$	215	5.13	5.95	3.58
Lungs	$f(t)$, g = $0.0360e^{(0.0669 - 0.000116t)t}$	238	37.9	25.7	5.14
Kidneys	$f(t)$, g = $0.00162e^{(0.0878 - 0.000167t)t}$	199	13.5	5.94	1.67
Liver	$f(t)$, g = $0.236e^{(0.0452 - 0.0000605t)t}$	211	42.2	20.1	10.8
Spleen	$f(t)$, g = $0.00160e^{(0.0722 - 0.000126t)t}$	211	3.22	1.68	2.05
Pancreas	$f(t)$, g = $0.0000641e^{(0.0643 - 0.000105t)t}$	224	0.65	0.43	-

[1]Day of gestation when the weight of the organ is equal to 50% of the predicted weight at parturition.
[2]Average values of cows from the current study. Pancreas weight was not evaluated in cows.

Table 13. Biometric measures of fetus in function of days of gestation and feeding level in pregnant Nellore cows

Item	Day of gestation				SEM	Feeding level[1]		SEM	P-value[2]		
	136	189	239	269		High	Low		FL	Day	Trt × day
Body length, cm	32.4[a]	52.6[b]	77.4[c]	86.6[d]	1.6	63.5	61.0	1.1	0.13	<0.01	0.25
Thoracic circumference, cm	24.0[a]	39.3[b]	57.6[c]	64.0[d]	1.1	46.5	46.0	0.8	0.70	<0.01	0.38
Height at shoulder, cm	23.3[a]	39.9[b]	65.5[c]	74.3[d]	1.1	51.5	49.9	0.7	0.14	<0.01	0.15
Height at rump, cm	21.9[a]	40.0[b]	64.9[c]	75.8[d]	0.9	50.9	50.3	0.7	0.52	<0.01	0.25
Cranial eyes circumference, cm	22.8[a]	34.6[b]	46.4[c]	50.0[d]	0.8	38.8	38.1	0.6	0.37	<0.01	0.41
Cranial neck circumference, cm	22.2	32.0	43.2	47.7	0.5	36.9	35.6	0.4	0.03	<0.01	0.04
High	22.0[a]	31.8[b]	44.1[cd]	49.5[e]	0.8	-	-	-	-	-	-
Low	22.4[a]	32.2[b]	42.2[c]	45.9[d]	0.8	-	-	-	-	-	-

[a-e]Within a variable, means differ (P < 0.10)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Table 14. Functions to predict fetus BW and fetal age from biometric measures of fetus[1]

Biometric predictor measure	Predicting feta age (d)			Predicting fetus BW		
	Function	r^2_{xy}	P-value	Function	r^2_{xy}	P-value
Body length, cm	$y = 14.3 \times BL^{0.6516}$	0.960	0.998	$y = 0.000155 \times BL^{2.701}$	0.981	0.708
Thoracic circumference, cm	$y = 16.89 \times TC^{0.6588}$	0.964	0.999	$y = 0.000263 \times TC^{2.769}$	0.974	0.864
Height at shoulder, cm	$y = 24.21 \times HS^{0.5536}$	0.975	0.999	$y = 0.001 \times HS^{2.367}$	0.975	0.985
Height at rump, cm	$y = 26.42 \times HR^{0.5322}$	0.983	0.999	$y = 0.00192 \times HR^{2.209}$	0.958	0.944
Cranial eyes circumference, cm	$y = 10.45 \times CEC^{0.8215}$	0.953	0.999	$y = 0.000041 \times CEC^{3.412}$	0.946	0.884
Cranial neck circumference, cm	$y = 9.97 \times CNC^{0.8478}$	0.970	0.995	$y = 0.000073 \times CNC^{3.314}$	0.962	0.689

[1]The r^2_{xy} value is for observed values regressed in function of predicted. P-value is the significance value for testing the joint hypothesis that $B_0 = 0$ and $B_1 = 1$ (Mayer et al., 1994), wherein P-values greater than 0.05 means that the H_0 hypothesis was accepted.

Table 15. Chemical composition of the fetus

Item	Day of gestation				Feeding level[1]		P-value[2]		
	136 (n = 8)	189 (n = 8)	239 (n = 8)	269 (n = 8)	High (n = 21)	Low (n = 23)	FL	Day	FL × day
N, g	17.2[a] ± 17.4	92[b] ± 17	380[c] ± 17	571[d] ± 17	273 ± 12	257 ± 12	0.36	<0.01	0.23
High	18.5 ± 23.9	97 ± 24	368 ± 24	608 ± 24	-	-	-	-	-
Low	15.9 ± 23.9	88 ± 24	392 ± 24	533 ± 24	-	-	-	-	-
Fat, g	12.2[a] ± 39.0	124[b] ± 39	606[c] ± 39	889[d] ± 39	422 ± 29	394 ± 27	0.49	<0.01	0.10
High	13.1 ± 54.9	129 ± 55	561 ± 55	984 ± 55	-	-	-	-	-
Low	11.3 ± 54.9	119 ± 55	652 ± 55	794 ± 55	-	-	-	-	-
Ash, g	28.6[a] ± 45.0	155[b] ± 45	650[c] ± 45	1,020[d] ± 45	458 ± 33	469 ± 31	0.80	<0.01	0.75
High	28.8 ± 63.0	165 ± 63	602 ± 63	1,035 ± 63	-	-	-	-	-
Low	28.5 ± 63.0	145 ± 63	697 ± 63	1,006 ± 63	-	-	-	-	-
Water, g	1,413 ± 642	5,392 ± 642	16,044 ± 642	21,297 ± 642	11,631 ± 472	10,442 ± 454	0.08	<0.01	0.05
High	1,432[a] ± 907	5,473[b] ± 907	16,067[c] ± 907	23,552[e] ± 907	-	-	-	-	-
Low	1,393[a] ± 907	5,311[b] ± 907	16,021[c] ± 907	19,042[d] ± 907	-	-	-	-	-
Energy, Mcal	0.67[a] ± 0.82	3.99[b] ± 0.82	16.8[c] ± 0.82	24.4[d] ± 0.82	11.7 ± 0.6	11.3 ± 0.3	0.61	<0.01	0.40
High	0.72 ± 1.16	4.22 ± 1.2	16.1 ± 1.2	25.7 ± 1.2	-	-	-	-	-
Low	0.62 ± 1.16	3.77 ± 1.2	17.5 ± 1.2	23.2 ± 1.2	-	-	-	-	-
N, g/kg	10.9 ± 0.3	14.5 ± 0.3	19.1 ± 0.3	21.1 ± 0.3	16.4 ± 0.2	16.4 ± 0.2	0.98	<0.01	0.01
High	11.5[a] ± 0.4	15.1[b] ± 0.4	18.6[c] ± 0.4	20.5[d] ± 0.4	-	-	-	-	-
Low	10.3[a] ± 0.4	14.0[b] ± 0.4	19.6[d] ± 0.4	21.8[e] ± 0.4	-	-	-	-	-
Fat, g/kg	7.78[a] ± 1.50	19.1[b] ± 1.5	30.5[c] ± 1.5	32.7[d] ± 1.5	22.3 ± 1.1	22.7 ± 1.1	0.79	<0.01	0.50
High	8.16 ± 2.13	19.8 ± 2.1	28.3 ± 2.1	33.1 ± 2.1	-	-	-	-	-
Low	7.40 ± 2.13	18.4 ± 2.1	32.7 ± 2.1	32.4 ± 2.1	-	-	-	-	-
Ash, g/kg	18.3 ± 1.1	24.4 ± 1.1	32.5 ± 1.1	37.8 ± 1.1	27.2 ± 0.8	29.3 ± 0.8	0.06	<0.01	0.04
High	18.1[a] ± 1.5	25.6[b] ± 1.5	30.4[c] ± 1.5	34.6[d] ± 1.5	-	-	-	-	-
Low	18.5[a] ± 1.5	23.2[b] ± 1.5	34.5[d] ± 1.5	40.9[e] ± 1.5	-	-	-	-	-

Water, g/kg	895 ± 3	853 ± 3	806 ± 3	786 ± 3	835 ± 2	835 ± 2	0.95	<0.01	0.01
High	890[f] ± 5	846[d] ± 5	812[c] ± 5	792[ab] ± 5	-	-	-	-	-
Low	899[f] ± 5	861[e] ± 5	800[b] ± 5	781[a] ± 5	-	-	-	-	-
Energy, Mcal/kg	0.42 ± 0.02	0.62 ± 0.02	0.84 ± 0.02	0.91 ± 0.02	0.69 ± 0.01	0.70 ± 0.01	0.59	<0.01	0.03
High	0.45[a] ± 0.03	0.65[b] ± 0.03	0.81[c] ± 0.03	0.87[c] ± 0.03	-	-	-	-	-
Low	0.40[a] ± 0.03	0.60[b] ± 0.03	0.87[c] ± 0.03	0.95[d] ± 0.03	-	-	-	-	-

[a-f]Within a variable, means differ (P < 0.10)

[1]High = HIGH-fed cows (16.0 ± 2.0 g of DM/kg of SBW) and Low = LOW-fed cows (10.8 ± 1.5 g of DM/kg of SBW).

[2]Probability values for effects of feeding level (FL), day of gestation (Day), and their interaction (FL × Day).

Figure captions

Figure 1. Position of fetal biometric measurements.

Figure 2. Relationship between fresh weights of the fetus (a), uterus (b), placenta (c), fetal fluids (d) and day of gestation in Nellore cows. Dotted lines represent extrapolation from data. For uterus and fetal fluids, solid line represent the single model for both HIGH-fed cows and LOW-fed cows cows.

Figure 3. Relationship between fresh weights of the fetus, uterus, placenta (all in the same scale) and day of gestation in Nellore cows. Except for uterus, solid lines represent the function for HIGH-fed cows cows and dash lines represent function for LOW-fed cows cows; dotted lines represent extrapolation from data. For uterus, solid line represent the single model for both HIGH-fed cows and LOW-fed cows cows.

Figure 4. Relationship between weights of the fetus, uterus and placenta, expressed as percentages of predicted weights at parturition and days of gestation. Except for uterus, solid lines represent the function for HIGH-fed cows cows and dash lines represent function for LOW-fed cows cows; dotted lines represent extrapolation from data. For uterus, solid line represent the single model for both HIGH-fed cows and LOW-fed cows cows.

Figure 5. Relationship between weights of fetus and placenta of Nellore cows

Figure 6. Relationship between weights of fetal organs, expressed as percentages of predicted weights at parturition and days of gestation. Black lines represent actual data range and dotted lines represent extrapolation from data.

Figure 7. Relationship between daily rate of gain of fetal organs as percentage of predicted weight at parturition and days of gestation. Black lines represent actual data range and dotted lines represent extrapolation from data.

Figure 1

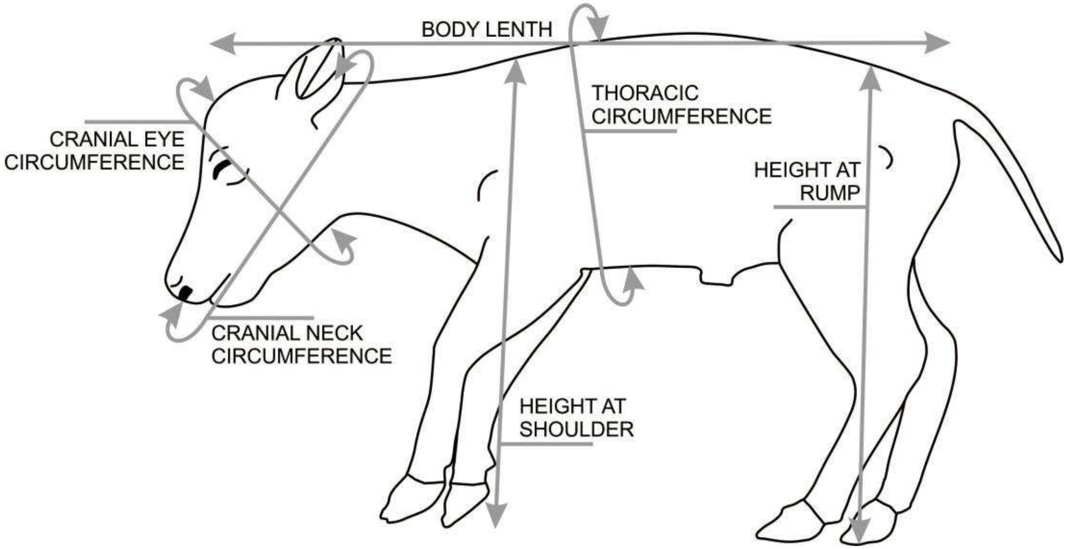
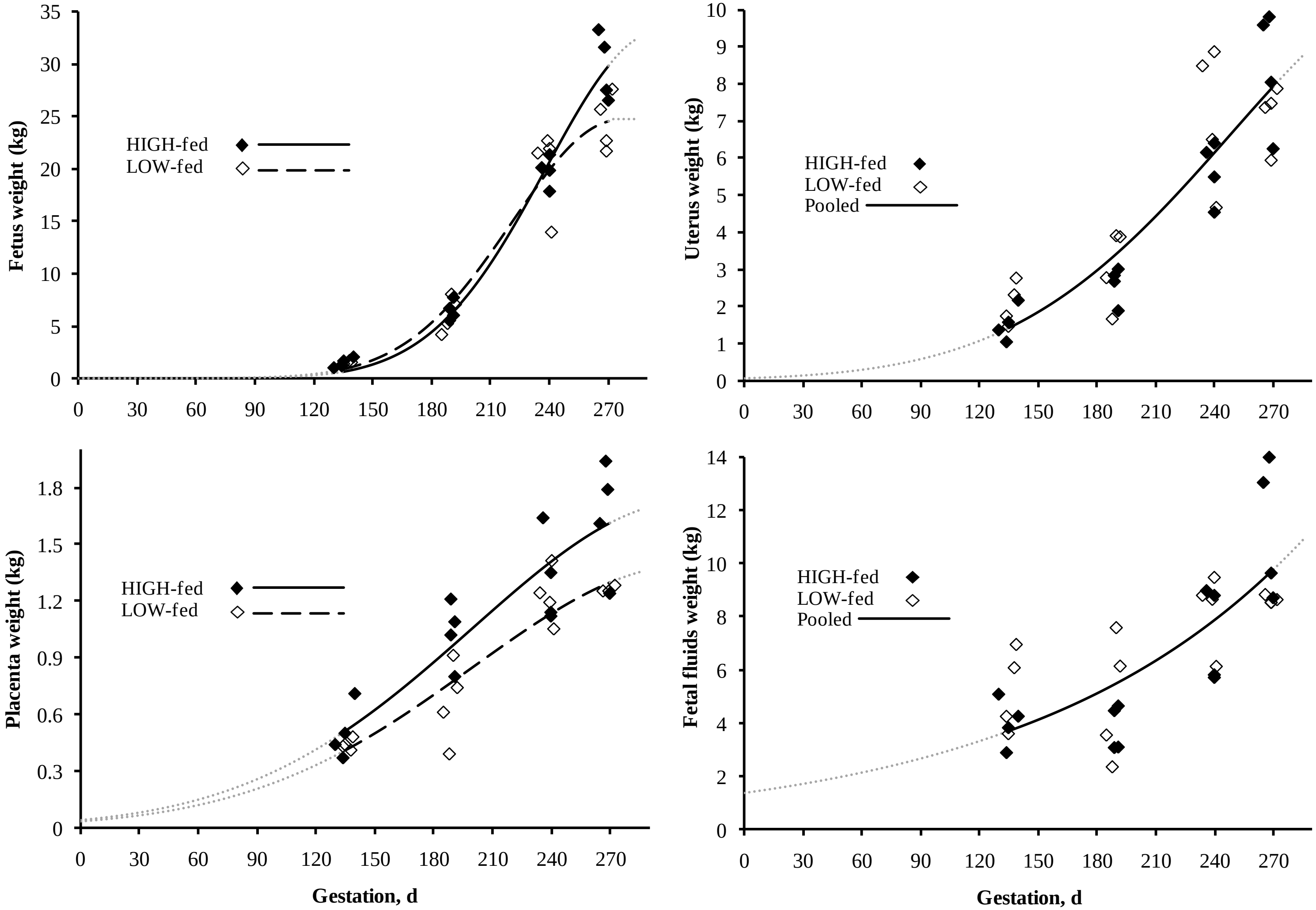
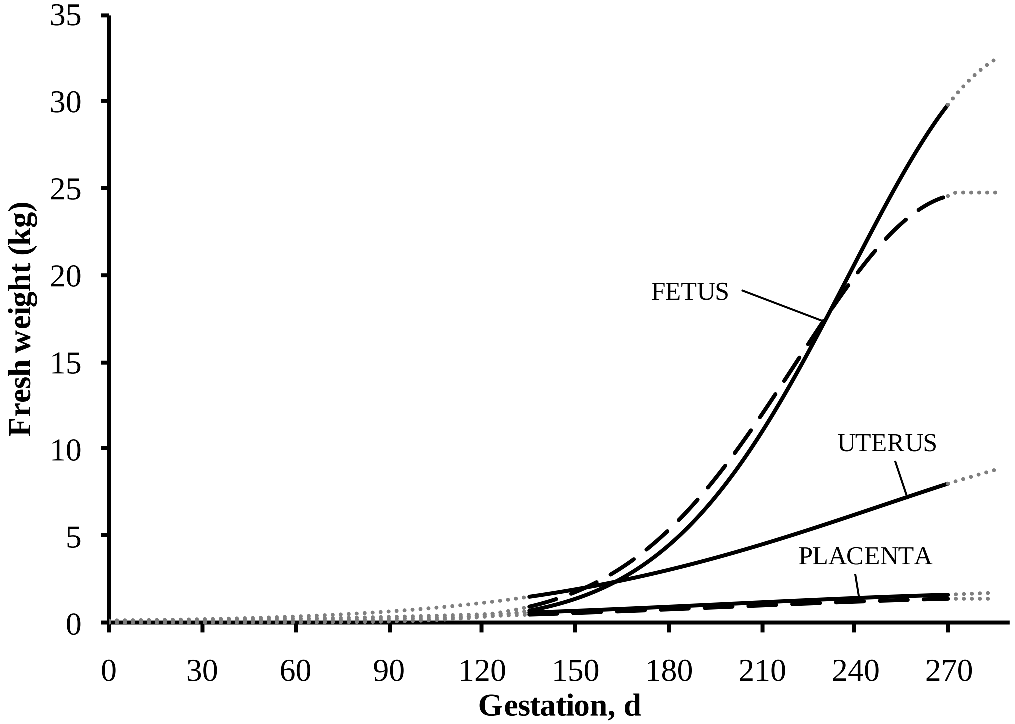

BODY LENTH
THORACIC
CIRCUMFERENCE
CRANIAL EYE
CIRCUMFERENCE
HEIGHT AT
RUMP
CRANIAL NECK
CIRCUMFERENCE
HEIGHT AT
SHOULDER

Figure 2



Fetus weight (kg)
HIGH-fed
LOW-fed
Uterus weight (kg)
HIGH-fed
LOW-fed
Pooled
Placenta weight (kg)
HIGH-fed
LOW-fed
Fetal fluids weight (kg)
HIGH-fed
LOW-fed
Pooled
Gestation, d
Gestation, d

Figure 3



35
30
25
20
15
10
5
0
Fresh weight (kg)
FETUS
UTERUS
PLACENTA
0
30
60
90
120
150
180
210
240
270
Gestation, d

Figure 4

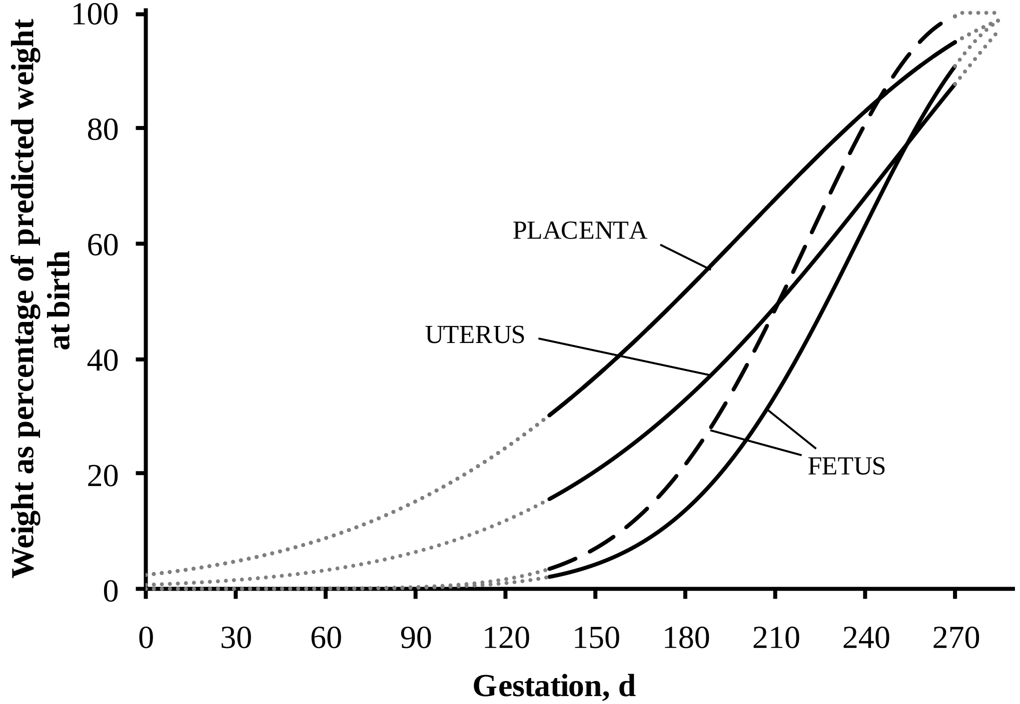
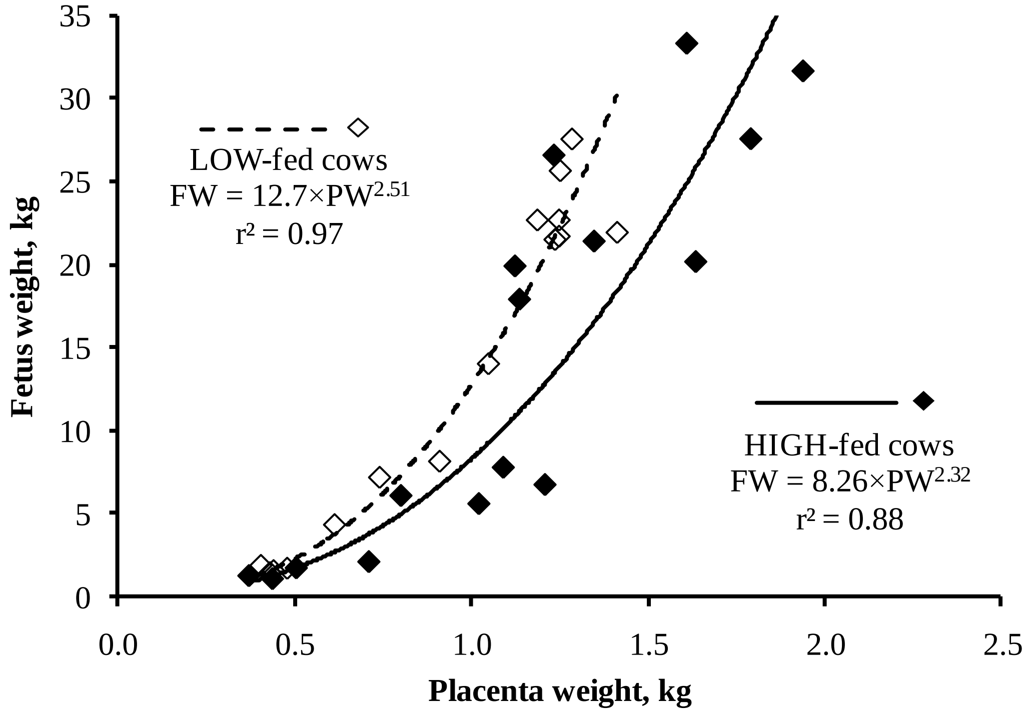
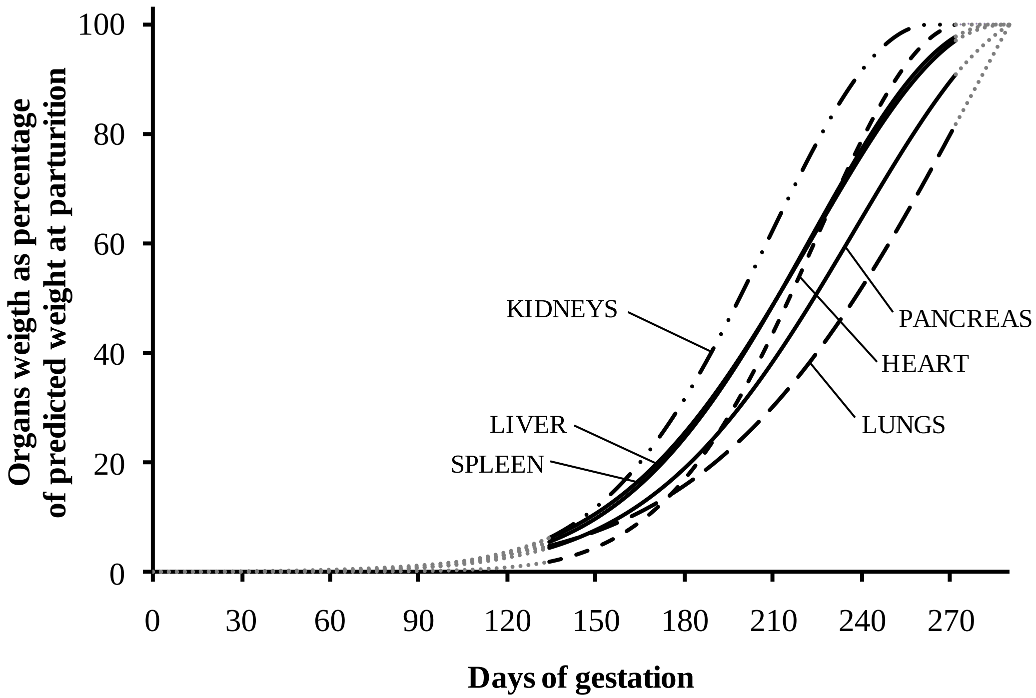

Weight as percentage of predicted weight at birth
100
80
60
40
20
0
PLACENTA
UTERUS
FETUS
0
30
60
90
120
150
180
210
240
270
Gestation, d

Figure 5



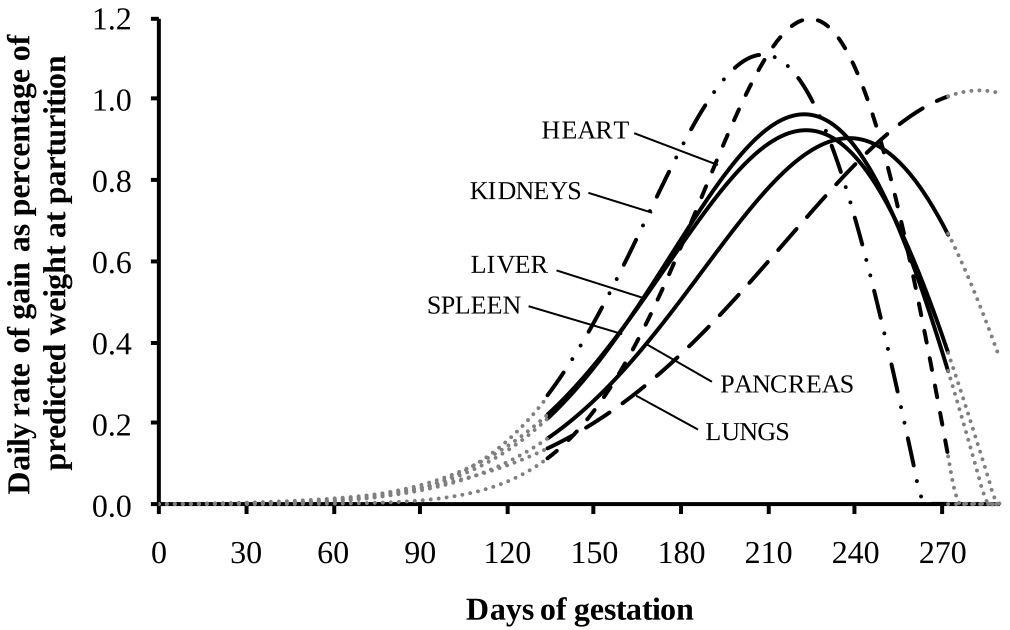
35
30
25
20
15
10
5
0
0.0
0.5
1.0
1.5
2.0
2.5
Fetus weight, kg
Placenta weight, kg
LOW-fed cows
FW = 12.7×PW2.51
r² = 0.97
HIGH-fed cows
FW = 8.26×PW2.32
r² = 0.88

Figure 6



Organs weigth as percentage of predicted weight at parturition
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
270
Days of gestation
KIDNEYS
LIVER
SPLEEN
PANCREAS
HEART
LUNGS

Figure 7


1.2
1.0
0.8
0.6
0.4
0.2
0.0
Daily rate of gain as percentage of predicted weight at parturition
HEART
KIDNEYS
LIVER
SPLEEN
PANCREAS
LUNGS
0
30
60
90
120
150
180
210
240
270
Days of gestation

CHAPTER 2

Achieving body weight adjustments for feeding status and pregnant or non-pregnant condition in beef cows

ABSTRACT: Dataset from 49 multiparous Nellore cows (32 pregnant and 17 non-pregnant) with average initial body weight of 451 ± 10 kg were used to develop a set of equations and relations for BW adjustments in pregnant or not pregnant beef cows. Cows were weighed every 28 d (0700 h, before feeding) to obtain the BW, and reweighed at the same time the following day after 16 h of fasting to obtain the shrunk body weight (**SBW**) values. Pregnant cows were separated into four groups of eight cows each and harvested at 136, 189, 239 and 269 d of pregnancy to obtain the empty body weight (**EBW**) and the weight of components related to pregnancy. To establish the relationships between the BW, SBW and EBW of pregnant and non-pregnant cows as a function of time of gestation, a set of general equations was tested, based on theoretical suppositions. The pregnant compound (**PREG**) was defined as the weight that is genuinely related to pregnancy, that includes the gravid uterus minus the non-pregnant uterus plus the accretion in udder related to pregnancy. The PREG was deducted from the SBW or EBW of a pregnant cow to estimate the non-pregnant SBW and EBW (**SBW_{np}** and **EBW_{np}**) and to calculate the body gain of only maternal tissues (**rSBG** and **rEBG**). The overall equation to predict SBW from BW was $0.8084 \times BW^{1.0303}$. The equation to predict gravid uterus weight in function of days of pregnancy (**DOP**) was $0.2106 \times e^{((0.03119 - 0.00004117 \times DOP) \times DOP)}$. There was no accretion of weight in udder up to 238 d of pregnancy and the accretion after 238 d was exponentially correlated with DOP. We conclude that the weight related to the pregnancy can be estimated in a live cow allowing estimating the non-pregnant EBW and SBW of a pregnant cow. This allows estimate and compare the weight gain of tissues not related to the pregnancy and the comparison of weight gain of a pregnant and non-pregnant cow.

KEYWORDS: beef cattle, *Bos indicus*, gestation, gravid uterus, shrunk body weight

RESUMO: Dados de 49 vacas Nelore multíparas (32 gestantes e 17 não gestantes) com peso médio inicial de 451 ± 10 kg foram utilizadas para desenvolver um grupo de equações e relações para ajuste de peso corporal em vacas gestantes e não gestantes, ampliando as opções para a pesquisa na área de gado de corte. A cada 28 dias as vacas foram pesadas pela manhã (7h, imediatamente antes da alimentação) para obter o peso vivo (PV), e repesadas ao mesmo horário no dia seguinte após 16 horas de jejum de sólidos para obter-se o peso vivo em jejum (PVJ). As vacas gestantes foram separadas em 4 grupos de 8 vacas cada e abatidas aos 136, 189, 239 e 269 dias de gestação para obter o peso de corpo vazio (PCVZ) e o peso dos componentes relacionados à gestação. Para estabelecer-se as relações entre PC, PCJ e PCVZ de vacas gestantes e não gestantes em função do tempo de gestação, um grupo de equações gerais foi avaliado, baseado em suposições teóricas que foram apresentadas com suas explanações. Sugeriu-se o conceito de componente gestação (PREG), que representa o útero grávido menos o peso do útero numa condição não gestante mais o acréscimo de úbere que ocorreu devido à gestação. A partir disso, o PREG foi subtraído dos valores de PVJ e PCVZ das vacas gestantes para estimar-se os PVJ e PCVZ não gestantes (PVJ_{np} e $PCVZ_{np}$) e para calcular-se o ganho de peso relativo apenas aos tecidos maternos (rGPVJ e rGPCVZ). A equação gerada para predizer PVJ a partir do PV foi $0{,}8084 \times BW^{1{,}0303}$. A equação gerada para predizer o peso do útero grávido em função dos dias de gestação (DG) foi $0.2106 \times e^{((0.03119\ -\ 0.00004117\ \times\ DG)\ \times\ DG)}$. Não foi observado acréscimo no peso do úbere até 238 dias de gestação e após isso o acréscimo foi exponencialmente correlacionado com DG. A aplicação das equações e relações geradas pode ser útil como uma ferramenta prática para separar a porção do ganho diário e peso vivo é referente à tecidos maternos e a porção relativa à gestação.

Palavras-chave: condição corporal, gestação, peso vivo, útero grávido, vacas de corte, Zebu

INTRODUCTION

The breeding herd accounts for about 70% of the total energy used in beef cattle production [1]. However, the number of studies investigating the production efficiency of breeding cows is lower than the number of studies involving the same aspect in other categories of beef cattle production system, such as growing and finishing animals [2]. One of the limiting factors for evaluating the production efficiency (e.g. feed efficiency) in mature cows is the difficulty to obtain reliable estimates of weight variation. This occurs due to the interaction of weight variation with specific physiological stages such as pregnancy and lactation.

In studies with pregnant cows both rate of body weight (**BW**) change and reproductive performance are commonly measured for assessment of the response of animals to experimental treatments. The deposition of body tissue reserves as well as fetal and uterine tissues contributes to the increase of BW of the cow leading to a complicated interpretation of the BW change. The comparison of the BW of a pregnant cow at the beginning and end of a study may not accurately represent the different physiological status because of the increased weight due to deposition of body tissue reserves or due to the growth of the components related to pregnancy, such as the gravid uterus and mammary gland. As such the standardization of BW of a cow in pregnant or non-pregnant condition the first step to meet their nutrient requirements [3].

It is also noteworthy that cattle are known to vary weight in function of feeding status (fasting or fed) throughout the day. Variation in gastrointestinal contents in ruminant animals is a major source of error in body weight gain measurements [4]. Weighing forms typically known are fed body weight (BW), shrunk body weight (**SBW**, weight after 14 to 16 hour fasting) and empty body weight (**EBW**, body weight without gastrointestinal content). The EBW is the weight information that presents the greatest correlation to carcass and animal traits [5], but can be directly acquired only after slaughter. Thus, the first step to estimate the nutrient requirements of cattle is to know the correct relationship between the BW, SBW and EBW. The Beef Cattle NRC system [6] adopted suggestions for weight adjustments among BW, SBW and EBW for growing animals and adult cows but not for pregnant cows. Moreover, the correction factors for the weight of adult cows based on physiological condition are minimal. Up to now there is no information with regard to the relationship between BW and SBW, and other relations and adjustments for live weight of pregnant cows for *Bos indicus* cattle.

There are limited number of studies reporting data about BW adjustments for pregnancy in cows [7, 8]. The available data did not consider a possible variation in the udder weight, which may increase in size as a function of pregnancy and consequently increases the weight of the cow. The use of equations and relationships to estimate the weight of the gravid and non-gravid uterus and the cow's udder as a function of gestational age and other characteristics allows the estimation of the live weight of cows and their body constituents, and subsequent weight comparison at various physiological stages and chronological sequences. Thus, we aimed to develop ways to estimate the actual weight of an adult cow, independent of feeding status (fasted or not) and in all stages of pregnancy. We hypothesized that there might be an increase in the udder weight as a function of gestation and we test at which point of gestation this increase is significant. Our results allows to know, in a live pregnant cow, which is the portion of weight that is from maternal tissues and which is from gestation tissues or tissues that increased in size due to pregnancy.

MATERIALS AND METHODS

Ethics Statement

All animal procedures were approved by the Animal Care and Use Committee of the Department of Animal Science of the Universidade Federal de Viçosa – Brazil (047/2012).

Animals

Forty-nine multiparous Nellore cows with average initial body weight of 451 ± 10 kg, age of 5.6 ± 0.5 years and body condition score of 4.4 ± 0.2 (1 to 9 scale) were used. Cattle were from an experiment reported by Duarte et al. [9]. From the initial 49 cows, 32 were randomly separated and hand mated with Nellore bulls to form the pregnant group. Twelve cows were randomly separated and assigned to the non-pregnant group and the five remaining cows were designated as the baseline.

Diet and management

Cows were housed in pens (48 m², 6 cows per pen) with a concrete floor, 15 m² of covered area, and *ad libitum* access to fresh water. Feed intake was measured individually using an electronic head gate system (Kloppen Soluções Tecnológicas, Pirassununga, SP, Brazil). Bred cows were at 47 ± 3 d of gestation when the feeding trial started.

All cows were fed the same diet twice daily (0700 and 1500 h) and divided into two groups according to level of feeding, either **HIGH** (*ad libitum*) or **LOW** (restricted feeding 1.2-times maintenance according to the Beef Cattle NRC [6]). Sixteen pregnant and 5 non-pregnant cows were fed HIGH level and 16 pregnant and 7 non-pregnant cows were fed the LOW-diet. The restricted feeding was estimated so that 1.2-times maintenance sustained pregnancy and the HIGH-fed allowed maternal tissue deposition. The diet consisted of corn silage, ground corn, soybean meal, urea and mineral mixture (Table 1).

Every 28 days cows were weighed in the morning (0700 h, before feeding) to obtain the BW, and reweighed at the same time the following day after 16 h of fasting to obtain the SBW values. Cattle had *ad libitum* access to water. Thus, the body gain (**BG**, kg/d) and the shrunk body gain (**SBG**, kg/d) were calculated.

The 32 pregnant cows were randomly assigned into 4 groups of 8 cows each (4 cows per each feeding level) and slaughtered at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. Non-pregnant cows were slaughtered at different times during the experiment (85 to 216 days of feeding control) to keep them in experiment for a similar period of time as the pregnant cows. Cows from the baseline group were harvested on day 0 of the experiment after the end of the adaptation period and were used to estimate the initial BW, SBW and EBW of the cows in the experiment.

Animal harvest and weighing of uterus and udder

Pre-slaughter animal care and handling procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products [10]. Before slaughter, feed was withheld from animals for 16 h, but they had *ad libitum* access to water the entire time. Slaughter was performed by using captive bolt stunning and exsanguination. The euthanasia of fetuses followed the American Veterinary Medical Association Guidelines [11].

Udders were removed along with the portion of hide that covered the udder and then weighed immediately after slaughter. The gravid and non-gravid uteri were sectioned at the cervix and weighed. The grouping of all whole body components after the wash of the digestive tract constituted the pregnant empty body weight (**EBW_p**, including udder and gravid uterus of pregnant cows) or the non-pregnant empty body weight (**EBW_{np}**, for non-pregnant cows).

Body condition score

Body condition scores (**BCS**) were assessed on a scale ranging from 1 = severely emaciated to 9 = very obese [12, 13] with 0.5 partial scoring and were determined by observation and palpation. The trial for determining the BCS of each cow was performed on the day of slaughter by two trained evaluators in a double-blind scheme. Each evaluator did not know the result of the evaluation of the other, and the average score was calculated. A new evaluation was performed if the scores of the two evaluators were further than 1.5 points apart.

Adjustment of cow BW measurements

To establish the relationships between the BW, SBW and EBW of pregnant and non-pregnant cows as a function of time of gestation, a set of general equations was tested, based on theoretical suppositions that are presented followed by their explanations (Table 2). In general, the pregnancy (referred mathematically as pregnancy component, **PREG**) was considered mathematically an extra component of the cow (cow BW + PREG). However, it was assumed that there is an interaction between the development of the specific tissues for the pregnancy (gravid uterus and udder) and the maternal tissues (lean, fat, viscera, etc). When appropriate, this interaction was considered and tested.

Multiple linear regressions (for Eq. A2, B2, F, I and J of Table 2), simple linear regressions (for Eq. A1, B1 and G of Table 2), or non-linear regressions were used to test the relationships between BS, SBW, and EBW. The intercept was used when biologically appropriate and statistically significant.

The basic mathematical model used to estimate the dynamics of gravid uterus (**GU**) in function of time of pregnancy was of the logistic form, similar to that suggested by Koong et al. [14]:

$$GU = GU_0 \times e^{(\beta_1 + \beta_2 \times DIP + \beta_3 \times BCS) \times DOP} \qquad \{1\}$$

where DOP = days of pregnancy, BCS = body condition score, GU_0 = weight of gravid uterus (kg) on day 0 of gestation and GU = the weight of gravid uterus (kg) on day DOP of pregnancy.

The mathematical model used to estimate the dynamics of udder as a function of time of gestation was based on what has previously reported by Fadel [15], as a segmented non-linear model with a period of staticity followed by an exponential model, as follow:

a. If DOP < β_0 then $UD = SBW_{np} \times \beta_1 \times BCS^{\beta_2}$

b. If DOP > β_0 then $UD = SBW_{np} \times \beta_1 \times BCS^{\beta_2} \times e^{[\beta_3 \times (DOP - \beta_0)]}$

{2}

where: DOP = days of pregnancy, UD = udder (kg), SBW_{np} = shrunk body weight of a cow in non-pregnant condition (kg) and BCS = body condition score. The parameter β_0 represents the moment in which the weight of the udder starts to increase as a function of days of pregnancy. The model "*a*" is used to estimate the weight of the udder of non-pregnant cows and the use of "*a*" or "*b*" model is used to pregnant cows depending on the values of B_0 and DOP.

Statistical Analysis

The REG and GLM functions in SAS version 9.2 (SAS Inst. Inc., Cary, NC) were used to estimate the regression parameters of linear functions. Parameters for nonlinear functions were estimated using the Gauss-Newton method in the NLIN procedure of SAS version 9.2. The value of 0.05 was adopted as critical level of probability for occurrence of Type I error.

Model evaluations

The predictive models were evaluated by the significance of the regression between the observed and predicted values [16], residual analysis, accuracy [Cb; 17], the mean square error of prediction [MSEP, 18] and the partition of sources of variation of MSEP [19]. These statistics were calculated using the Model Evaluation System (MES, v.3.0.1, http://nutritionmodels.tamu.edu/mes.htm) [20]. In addition, the Akaike Information Criteria [AIC; 21] was calculated according to Motulsky and Christopoulos [22].

RESULTS AND DISCUSSION

Estimation of SBW from BW

The BW and days of pregnancy (**DOP**) were significant (*P*<0.05) to estimate the SBW of pregnant and non-pregnant cows when a multiple linear regression was used (Table 2, Eq. A1 and A2). However, less than 0.5% of the variation in the SBW of the pregnant cows can be explained by DOP. The AIC was greater when the SBW and DOP were used in the model (AIC = 263.4) than when only SBW was used (AIC = 258.4). The theoretical models presented in the Eq. A3 and A4 (Table 2) cannot be practically developed because the estimative of PREG is dependent on the SBW value (Table 2, Eq. C, D and F), making it impractical to consider a possible interaction between the SBW/BW ratio and the time of pregnancy. Thus, we decided to use only BW to estimate SBW of pregnant and non-pregnant cows, assuming that the effect of DOP is negligible.

The equations generated to predict the SBW only as a function of the BW (Eq. {3} and {4}) had high Cb values (0.999 for Eq. {3} and 1.000 for Eq. {4}) when the estimated and observed data were compared. However, the hypothesis that $\beta_0 = 0$ and $\beta_1 = 1$ was rejected (P=0. 003) for Eq. {3} when the parameters were analyzed. For the model presented in Eq. {4}, this hypothesis was accepted (P = 0.889). Equation {4} also has a lower MSEP (23.6) than the linear model (28.9). Moreover, the decomposition of the MSEP shows that only 0.06 (0.3%) of the MSEP value originated from the systematic errors in the power model (Eq. {4}), whereas in the linear model this value is 4.62 (15.9% of the MSEP). The AIC also shows a better value for the power model in comparison with the linear model (250.5 and 258.4, respectively). This difference indicates that, according to the AIC theory [based on the AIC evidence ratio; 22], there is a 98.2% of probability that the model given by Eq. {4} is better than that from Eq. {3}. This means that the model Eq. {4} of is 53-times more likely to be correct than the Eq. {3} model.

$$SBW = 0.9763\ (\pm 0.0013) \times BW \qquad \{3\}$$

$$SBW = 0.8084\ (\pm 0.0350) \times BW^{1.0303\ (\pm 0.0069)} \qquad \{4\}$$

where SBW = shrunk body weight (kg) and BW = body weight (kg).

These results suggest the utilization of a nonlinear model to estimate the SBW value for pregnant and non-pregnant cows. The value of the BW exponent greater than 1 (1.0303) shows an increase in the value of the relationship between SBW and BW as body weight increases. Biologically, it suggests that the decrease of weight caused by fasting is lower in proportion to the whole body weight, as BW increases. The relationship between SBW and BW calculated using the Eq. {4} for a 300-kg BW cow is 0.961, very near to the value suggested by the Beef Cattle NRC [6], which is 0.96. However, for a 700kg BW cow this relationship is equal to 0.986.

Gravid uterus weight

Theoretically, the weight of the GU would be a function of size of the cow (SBW), the feeding level, and the time of pregnancy. The inclusion of SBW in the model is unworkable, because the weight of the GU is being estimated to estimating the cow SBW. Likewise, although differences have been observed in the weight of the GU at 270 days of gestation as a function of feeding level with the same cows used in this study [2]; the inclusion of feeding level in the model is impractical because the feeding level is quite a variable concept. If feeding level refers to daily intake of DM, there may be differences in energy concentration among diets. If feeding level is referred to in relation to the maintenance level, there is still unclear information on the maintenance of Zebu beef cows. Thus, we decided to use of BCS as it is easy to measure and indirectly includes information about cow size and feeding level. When the DOP and BCS where included in the model it was better fitted to the data than when DOP and feeding level were included (AIC = 52.0 and 54.7; MSEP = 23.6 and 28.8, for inclusion of DOP and BCS or DOP and feeding level, respectively). In case of no availability of the BCS, a model to predict the GU as function only of DOP was also generated (Eq. {6}).

$$GU = 0.2243\ (\pm 0.3099) \times BCS^{0.3225\ (\pm 0.1331)} \times e^{((0.02544\ (\pm 0.0125) - 0.0000286\ (\pm 0.000028) \times DOP) \times DOP)} \quad \{5\}$$

$$GU = 0.2106\ (\pm 0.3238) \times e^{((0.03119\ (\pm 0.0137) - 0.00004117\ (\pm 0.00003) \times DOP) \times DOP)} \quad \{6\}$$

where GU = gravid uterus (kg), DOP = days of pregnancy and BCS = body condition score. The Table 3 shows examples of weights of GU estimated by Eq. {5} and Eq. {6} for cows with different BCS in different times of pregnancy.

Non-pregnant uterus and ovaries weight

A linear model without intercept showed the best fit (based on AIC value) for describing the weight of the uterus plus ovaries of non-pregnant cows (**UT_{np}**, Eq. {7}). The same model can be used to estimate the UT_{np} of pregnant cows. However, an adjustment to the value of SBW (Eq. {8}) is required, which is an adaptation for a provisional value of SBW_{np} of pregnant cows as there is a circular reference at this point due to the fact that the estimate of UT_{np} is part of the estimates made to obtain the real value of SBW_{np}.

$$UT_{np} = 0.0012\ (\pm 0.00005) \times SBW_{np} \quad \{7\}$$

$$\text{If } DOP \leq 240 \text{ then } UT_{np} = 0.0012\ (\pm 0.00005) \times (SBW_p - GU + 0.6)$$
$$\text{If } DOP > 240 \text{ then } UT_{np} = 0.0012\ (\pm 0.00005) \times (SBW_p - GU + 0.6 - 2) \quad \{8\}$$

where UT_{np} = uterus plus ovaries of a non-pregnant cow (kg), SBW_{np} = non-pregnant shrunk body weight, SBW_p = pregnant shrunk body weight, 0.6 = the value of a UT_{np} of a 500-kg cow and 2 = the average value for udder accretion due to pregnancy when pregnancy is longer than 240 days. The coefficients of 0.6 and 2 were used because it is not possible at this point to estimate the exact value of SBW_{np} for a pregnant cow. Thus, they were used as estimates to produce the minimum error as possible.

Accretion of weight as gravid uterus during pregnancy

From the values of GU and UT_{np} the accretion of weight as gravid uterus during gestation (GU_{dp}) can be estimated according to Eq. D of Table 2. As an example, a 500-kg BW pregnant cow, BCS of 5 and 200 days of pregnancy, the calculation of GU_{dp} is as follows:

$$GU = 0.2243 \times 5^{0.3225} \times e^{((0.02544 - 0.0000286 \times 200) \times 200)} = 19.44 \text{ kg}$$

$$GU_{dp} = GU - UT_{np}$$

$$GU_{dp} = 19.44 - 0.0012 \times (500 - 19.44 + 0.6)$$

$$GU_{dp} = 18.86 \text{ kg}$$

Udder modeling

During pregnancy, especially near parturition, changes occur in the bovine mammary gland, through the formation of milk secretory tissue. Although this phenomenon commonly occurs in heifers, this was also observed in multiparous cows used in the current study. The udder weight data showed an increase in the weight from 240 days of pregnancy (Figure 1). The non-linear logistic model (Eq. {1}) did not fit to the data of udder weights well as a function of DOP. Thus, a non-linear segmented model (Eq. {2}), as presented by Fadel [15], was used to describe the dynamics of the udder in function of gestation. The model that showed better fit was with the inclusion of SBW, BCS and DOP for pregnant cows and SBW and BCS for non-pregnant cows (Eq. {9}). The intercept of the model was 238 indicating no significant increase in the weight of the cow's udder at this period of pregnancy. From this period, there is an exponential increase in udder weight due to the formation of parenchyma. Similarly to the weight of the gravid uterus, the feeding level was not included, but is indirectly represented by the BCS. The SBW_{np} was included in this model because it represents much of the variation in the weight of the udder. As previously observed there is also a case of a circular reference, and to estimate the SBW_{np} of pregnant cows it is suggested to use $SBW_{np} = SBW_p - GU + 0.6$ (and - 2 if DOP is greater than 240 days).

$$\text{If DOP} \leq 238 \text{ then } UD_{np} = SBW_{np} \times 0.00589\ (\pm 0.00192) \times BCS^{0.2043\ (\pm 0.1809)}$$
$$\text{If DOP} > 238 \text{ then } UD_p = UD_{np} \times e^{((DOP - 238) \times 0.0109\ (\pm 0.0019))} \quad \{9\}$$

where UD_{np} = udder of a non-pregnant cow (kg), SBW_{np} = shrunk body weight of a non-pregnant cow (kg), BCS = body condition score, DOP = days of pregnancy, UD_p = udder of a pregnant cow (kg).

The high variability of udder weight among the cows complicates the modeling of factors that affect the weight of this component in beef cows. The model presented in Eq. {9} showed the best fit among other tested and so was used. When the observed values were regressed in function of predicted values, a Cb = 0.9926 and MSEP = 0.642 were obtained, being 92.8% of MSEP variations from random sources. The hypothesis that $\beta_0 = 0$ and $\beta_1 = 1$ was tested according to Mayer et al. [16] and was accepted (P=0.209), indicating that the model is appropriate.

Based on the model presented in Eq. {9} can be defined the increase in udder in function of pregnancy (**UD_{dp}**, Eq. H in Table 2) as follows:

$$\text{If DOP} \leq 238 \text{ than } UD_{dp} = 0$$
$$\text{If DOP} > 238 \text{ than } UD_{dp} = UD_{np} \times e^{((DOP - 238) \times 0.0109\ (\pm 0.0019))} - UD_{np} \quad \{10\}$$

where DOP = days of pregnancy and UD_{dp} = udder accretion due to pregnancy. In practice, it is suggested the adoption of 240 days of pregnancy as the time in which the udder starts to increase due to pregnancy.

Pregnancy compound

Based on the Eq. C of the Table 2, the pregnant compound (**PREG**) can be defined as Eq. {6} – Eq. {8} + Eq. {10}, as follow:

$PREG = GU_{dp} + UD_{dp}$

$PREG = GU – UT_{np} + UD_{dp}$

If the pregnancy is less than 240 days the value of UD_{dp} must be excluded.

As an example, a pregnant cow with 550 kg of SBW_p, BCS of 6, at 270 d of pregnancy, the BW that is attributable to the pregnancy can be calculated as follows:

$PREG = GU_{dp} + UD_{dp}$

$GU_{dp} = GU – UT_{np}$

$GU = 0.2243 \times BCS^{0.3225} \times e^{((0.02544 – 0.0000286 \times DOP) \times DOP)}$

$GU = 0.2243 \times 6^{0.3225} \times e^{((0.02544 – 0.0000286 \times 270) \times 270)}$

$GU = 47.8$ kg

$GU_{dp} = GU - 0.0012 \times (SBW_p – GU + 0.6 – 2)$

$GU_{dp} = 47.8 – 0.0012 \times (550 – 47.8 + 0.6 – 2)$

$GU_{dp} = 47.2$ kg

$UD_{dp} = UD_{np} \times e^{((DOP – 238) \times 0.0109} – UD_{np}$

$UD_{np} = SBW_{np} \times 0.00589 \times BCS^{0.2043}$

$UD_{np} = (550 – 47.8 + 0.6 – 2) \times 0.00589 \times BCS^{0.2043}$

$UD_{np} = 4.25$ kg

$UD_{dp} = 4.25 \times e^{((270 – 238) \times 0.0109} – 4.25$

$UD_{dp} = 1.76$ kg

$PREG = 47.2 + 1.76$ kg

$PREG = 48.98$ kg

Estimation of non-pregnant SBW of a pregnant cow

The knowledge of the values of SBW_p and PREG allows the estimation of the SBW_{np} values according to Eq. A4 of Table 2. For the cow in the previous example, the SBW_{np} value is: 550 – 48.98 = 501.02 kg. Therefore, in this case tissues related to pregnancy represented 8.9% of cow's weight.

Estimation of EBW from SBW

The fit of models to predict EBW from SBW was performed in a dataset that grouped the data from non-pregnant cows with SBW and EBW data of pregnant cows, from which was deducted the value of PREG, estimated as described above.

The two equations generated to predict the EBW_{np} as a function of the SBW_{np} (Eq. {11} and {12}) have a Cb value of 0.999 for Eq. {11} and 1.000 for Eq. {12} when the estimated and observed data were compared. The hypothesis that $\beta_0 = 0$ and $\beta_1 = 1$ was accepted for both equations (P=0.848 for Eq. {11} and P=0.999 for Eq. {12}). Equation {12} has a little lower MSEP (91.9) than the linear model (92.8). The decomposition of the MSEP shows that 99.2 and 100% of the MSEP value originates from random errors in Eq. {11} and Eq. {12}, respectively. The AIC was 98.4 for the Eq. {11} model and 100.2 for the Eq. {12} model. These results show that both models can be safely used to predict EBW_{np}, although we suggest the use of the power model (Eq. {12}) to have greater biological sense. A value of the SBW_{np} exponent greater than 1 (1.0122) means that there is an increase in the value of the relationship between EBW_{np} and SBW_{np} as body weight increases. This is biologically plausible and suggests that larger animals have a lower proportion of weight on the content of the gastrointestinal tract by having logically lower proportion of the gastrointestinal tract.

$$EBW_{np} = 0.9092\ (\pm 0.0028) \times SBW_{np} \qquad \{11\}$$

$$EBW_{np} = 0.8424\ (\pm 0.1025) \times SBW_{np}^{1.0122\ (\pm 0.0195)} \quad \{12\}$$

where EBW_{np} = empty body weight of a non-pregnant cow (kg) and SBW_{np} = shrunk body weight of a non-pregnant cow (kg). A graphic representation of the relation among EBW_{np} and SBW_{np} is shown in Figure 2.

It can be observed that the relationship between EBW_{np} and SBW_{np} has a low variability between animals from different sizes or different feeding systems. The Beef Cattle NRC [6] and BR-CORTE [23] suggest the use of relations between EBW and SBW for growing animals of 0.891 and 0.895, respectively, similarly to those observed in the current study for adult cows. The relation between EBW_{np} and SBW_{np} calculated using Eq. {12} for a 300-kg cow is 0.9031 and for a 700-kg cow is 0.9125.

From the relationship between EBW_{np} and SBW_{np}, the relationship between EBW_p and SBW_p can also be estimated. For this, it is necessary to deduct the value of PREG from these values. Based on Eq. B3 and Eq. B4 of Table 2, and Eq. {12}, the relationship between EBW_p and SBW_p can be calculated as follows:

$EBW_p = EBW_{np} + PREG$

$EBW_{np} = 0.8424 \times SBW_{np}^{1.0122}$

$SBW_{np} = SBW_p - PREG$, thus

$EBW_p = (0.8424 \times (SBW_p - PREG)^{1.0122}) + PREG$

The relationship consists of deducting the PREG component from the analysis, considering it as a separate portion of the weight of the cow. Based on the equations above the relationship between EBW_p and EBW_{np}, and EBW_p and SBW_{np} can also be established.

Practical usage of BW adjustments in pregnant cows

A summary of the equations and relationships generated to adjust the weights of pregnant cows to a non-pregnant status and also to establish the relationship between BW, EBW and SBW in pregnant or non-pregnant beef cows is presented in Table 4. The application of the equations in Table 4 can be a useful tool to separate how much of the total gain is attributable to maternal tissues and how much is from the pregnancy. Although the equations and relationship shown in Table 4 were made using *B. indicus* cattle it can be adapted for *B. taurus* cattle replacing some equations by those generated using *B. taurus* cattle: Eq. {4} should be replaced by the factor 0.96 used in Beef Cattle NRC [6]; Eq. {12} and {13} should be replaced by factor 0.851 used by Beef Cattle NRC [6] for mature cows; Eq. {5} and {6} should be replaced by the model generated by Ferrell et al. [24] to predict the weight of gravid uterus of pregnant *B. taurus* heifers [$GU (g) = 743.9 \times e^{(0.02 - 0.0000143 \times DOP) \times DOP}$].

A Microsoft Excel spreadsheet was constructed based on the equations presented in Table 4 and considerations made in this topic on BW adjustment for *B. taurus* cows. The spreadsheet is a supporting information of this paper.

Problems related to BW adjustments in pregnant cows

It is not possible to make all the proposed weight adjustments without incurring some errors. One of the first problems is the fact that there are some circular references in the theory of the proposed equations. To try to circumvent this problem we used several fixed parameters, estimated for the average of the sample of animals used to generate the proposed equations.

Another key point to consider is that the growth of the components related to the pregnancy and maternal tissues does not occur independently. During pregnancy changes occur in the maternal tissues to support nutrition and growth of the gravid uterus. Reduction in the size of the viscera [2, 25-28] and mobilization of maternal body reserves tissues [29] can be caused by a cow during pregnancy, although it is still a contradictory issue.

CONCLUSIONS

The live weight of pregnant cows can be adjusted for non-pregnant condition by deduction of uterus and udder accretion weight due to pregnancy, which can be estimated as a function of days of pregnancy and shrunk body weight. Udder weight accretion as a function of pregnancy occurs after 238 d of pregnancy in Nellore cows.

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REFERENCES

1. Ritchie RW (1995) The optimum cow – what criteria must she meet? . In: B. I. Federation, editor editors. RESEARCH SYMPOSIUM AND ANNUAL MEETING, 27. Kansas City: Beef Improvement Federation. p.126-145.
2. Gionbelli MP (2013) NUTRIENT REQUIREMENTS AND QUANTITATIVE ASPECTS OF GROWTH, DEVELOPMENT AND DIGESTION OF PREGNANT AND NON-PREGNANT NELLORE COWS. PhD Thesis.

Department of Animal Science. Viçosa: Universidade Federal de Viçosa. pp. 198.

3. PAULINO PVR, FONSECA MA, HENRIQUES LT, VALADARES FILHO SC and DETMANN E (2010) Nutritional Requirements of Nellore Cows and Calves. In: S. C. Valadares Filho, M. I. Marcondes, P. V. R. Paulino and M. L. Chizzotti, editors. Nutrient requirements of Zebu cattle - BR-CORTE. Visconde do Rio Branco: Suprema Gráfica e Editora. pp. 167-185.
4. Lofgreen GP, Hull JL and Otagaki KK (1962) Estimation of the empty body weight of beef cattle. J Anim Sci 21: 20.
5. Fox DG, Dockerty TR, Johnson RR and Preston RL (1976) Relationship of empty body weight to carcass weight in beef cattle. J Anim Sci 43: 566-568.
6. NRC (2000) Nutrient Requirements of Beef Cattle. Washington, DC: National Academy Press. 242 p.
7. Silvey MW and Haydock KP (1978) A note on live-weight adjustment for pregnancy in cows. Animal Production 27: 113-116.
8. Williams WT and Edye LA (1975) The analysis of reproductive records with use of labelled sequences, and its application to a grazing experiment. Australian Journal of Agricultural Research 26: 665-672.
9. Duarte MS, Gionbelli MP, Paulino PVR, Serão NVL, Silva LHP, et al. (2013) Effects of pregnancy and feeding level on carcass and meat quality traits of Nellore cows. Meat Science 94: 139-144.
10. Brasil (1997) Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal [*Regulation of Industrial and Sanitary Inspection of Animal Products*]. Brasília, DF: Ministério da Agricultura Pecuária e Abastecimento.

11. AVMA (2013) Guidelines for the Euthanasia of Animals: 2013 Edition. Schaumburg: American Veterinary Medical Association. 102 p.
12. Nicholson MJ and Butterworth MH (1986) A guide to condition scoring of zebu cattle. International Livestock Centre for Africa, Addis Ababa.
13. Richards MW, Spitzer JC and Warner MB (1986) Effect of Varying Levels of Postpartum Nutrition and Body Condition at Calving on Subsequent Reproductive Performance in Beef Cattle. J Anim Sci 62: 300-306.
14. Koong LJ, Garrett WN and Rattray PV (1975) A Description of the Dynamics of Fetal Growth in Sheep. J Anim Sci 41: 1065-1068.
15. Fadel JG (2004) Technical note: Estimating parameters of nonlinear segmented models. J Dairy Sci 87: 169-173.
16. Mayer DG, Stuart MA and Swain AJ (1994) Regression of real-world data on model output: an appropriate overall test of validity. Ag Syst 45: 93-104.
17. Lin LIK (1989) A concordance correlation coefficient to evaluate reproducibility. Biometrics 45: 255-268.
18. Bibby J and Toutenburg H (1977) Prediction and improved estimation in linear models. Berlin, Germany: John Wiley & Sons. 188 p.
19. Theil H (1961) Economic forecasts and policy. In: R. Strotz, J. Tinbergen, P. J. Verdoorn and H. J. Witteveen, editors. Contributions to Economic Analysis. Amsterdam: North-Holland Publishing Company. pp. 6-48.
20. Tedeschi LO (2006) Assessment of the adequacy of mathematical models. Ag Syst 89: 225-247.
21. Akaike H (1974) A new look at the statistical model indentification. IEEE transactions on Automatic Control AC-19: 716-723.

22. Motulsky H and Christopoulos A (2003) Fitting Models to Biological Data using Linear and Nonlinear Regression. San Diego, CA: GraphPad Software Inc.
23. Valadares Filho SC, Marcondes MI, Chizzotti ML and Paulino PVR (2010) Nutrient Requirements of Zebu Beef Cattle - BR-CORTE. Viçosa: Suprema Gráfica e Editora. 185 p.
24. Ferrell CL, Garrett WN and Hinman N (1976) Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. J Anim Sci 42: 1477-1489.
25. Meyer AM, Reed JJ, Vonnahme KA, Soto-Navarro SA, Reynolds LP, et al. (2010) Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. J Anim Sci 88: 2410-2424.
26. Caton JS, Reed JJ, Aitken RP, Milne JS, Borowicz PP, et al. (2009) Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J Anim Sci 87: 222-235.
27. Scheaffer AN, Caton JS, Bauer ML and Reynolds LP (2001) Influence of pregnancy on body weight, ruminal characteristics, and visceral organ mass in beef heifers. J Anim Sci 79: 2481-2490.
28. Scheaffer AN, Caton JS, Redmer DA and Reynolds LP (2004) The effect of dietary restriction, pregnancy, and fetal type in different ewe types on fetal weight, maternal body weight, and visceral organ mass in ewes. J Anim Sci 82: 1826-1838.

29. McNeill DM, Slepetis R, Ehrhardt RA, Smith DM and Bell AW (1997) Protein requirements of sheep in late pregnancy: partitioning of nitrogen between gravid uterus and maternal tissues. J Anim Sci 75: 809-816.

FIGURE LEGENDS

Figure 1. Relationship between days of pregnancy and weight of fresh udder in Nellore cows. The continuous line represents the estimation of the weight of fresh udder for a cow with the average shrunk body weight and body condition score (494 kg and 5.6, respectively) of the cows used in this study.

Figure 2. Relationship among non-pregnant shrunk body weight and non-pregnant empty body weight in Nellore cows. The continuous line represents the estimation of non-pregnant empty body weight from non-pregnant shrunk body weight using Eq. {12.

TABLES

Table 1. Ingredients and chemical composition of the diet

Item	Silage	Concentrate	Diet
Ingredient , % of DM			
Corn Silage	100.0	-	84.3
Ground corn	-	54.6	8.5
Soybean meal	-	33.0	5.1
Urea	-	7.3	1.2
Sodium chloride	-	2.1	0.37
Ammonium sulfate	-	1.5	0.25
Dicalcium phosphate	-	1.3	0.23
Microminerals mixture[1]	-	0.17	0.028
Analyzed composition[2], %			
DM	28.0	89.2	37.6
OM	94.7	92.8	94.4
CP	7.8	44.1	13.5
EE	2.9	2.5	2.8
NDF_{ap}	45.8	8.2	39.9
iNDF	20.8	0.65	17.6
NDIN	38.2	7.0	11.4
NFC	38.2	53.0	40.6
TDN	-	-	66.6
GE (Mcal/kg)	3.82	3.49	3.77

[1]Zinc sulfate (56.3 %), manganese sulfate (26.2 %), copper sulfate (16.8 %), potassium iodate (0.37 %), cobalt sulfate (0.23 %) and sodium selenite (0.10 %).
[2]NDF_{ap} = neutral detergent fiber corrected to ash and protein, iNDF = indigestible neutral detergent fiber and NFC = non fibrous carbohydrates.

Table 2. Set of general theoretical assumptions used to establish cows BW adjustments

Code	Model	What it means
A1	$SBW_{np} = f(BW_{np})$	SBW of a non-pregnant cow (**SBW_{np}**) is function of the BW of a non-pregnant cow (**BW_{np}**)
A2	$SBW_{p} = f(BW_{p}, DOP)$	SBW of a pregnant cow (**SBW_{p}**) is function of BW of a pregnant cow (**BW_{p}**) and days of pregnancy (**DOP**)
A3	$SBW_{p} = SBW_{np} + PREG$	SBW_{p} also can be expressed as the SBW of the cow if in a non-pregnant condition plus the increase of weight occurred due to the pregnancy, called pregnancy compound (**PREG**). For this we need to consider non interaction between the ratio BW/SBW and DOP
A4	$SBW_{np} = SBW_{p} - PREG$	SBW_{np} if the cow is pregnant can be estimated as the SBW_{p} minus PREG. Is the inverse of the equation A3
B1	$EBW_{np} = f(SBW_{np})$	EBW of a non-pregnant cow (**EBW_{np}**) is function of SBW of a non-pregnant cow (**SBW_{np}**)
B2	$EBW_{p} = f(SBW_{p}, DOP)$	EBW of a pregnant cow (**EBW_{p}**) is function of SBW_{p} and DOP
B3	$EBW_{p} = EBW_{np} + PREG$	EBW_{p} also can be expressed as the EBW of the cow if in a non-pregnant condition plus the pregnancy compound. For this we need to consider that when discounted the PREG, the relation between SBW and EBW is equal for pregnant and non-pregnant cows
B4	$EBW_{np} = EBW_{p} - PREG$	EBW_{np} if the cow is pregnant can be estimated as the EBW_{p} minus PREG. Is the inverse of the equation B3
C	$PREG = GU_{dp} + UD_{dp}$	PREG means the all tissues increase due to the pregnancy and is equal to the GU accretion during the pregnancy (**GU_{dp}**) plus udder accretion during the pregnancy (**UD_{dp}**)
D	$GU_{dp} = GU - UT_{np}$	GU_{dp} is equal to GU minus the weight of the uterus of the cow in non-pregnant condition (**UT_{np}**)
E	GU = fetus + amniotic fluid + placenta + uterus + ovaries	Gravid uterus (**GU**) is equal to the sum of its compounds
F	$GU = f(SBW, LF, DOP)$	GU is function of SBW, level of feeding (**LF**) and DOP
G	$UT_{np} = f(SBW)$	UT_{np} is function of SBW
H	$UD_{dp} = UD_{p} - UD_{np}$	UD_{dp} is equal to the weight of udder of a pregnant cow (**UD_{p}**) minus the udder weight of the cow in a non-pregnant condition (**UD_{np}**)
I	$UD_{p} = f(SBW, LF, DOP)$	UD_{p} is function of SBW, LF and DOP
J	$UD_{np} = f(SBW, LF)$	UD_{np} is function of SBW and LF

Table 3. Examples of the estimation of gravid uterus weight as a function of days of pregnancy (Eq. {5) or days of pregnancy and body condition score (Eq. {6)

Equation	Body condition score		
	3	5	7
		135 d in pregnancy	
{5	6.71	6.71	6.71
{6	5.88	6.94	7.73
		270 d in pregnancy	
{5	47.6	47.6	47.6
{6	38.2	45.0	50.2

Table 4. Summary of equations used to adjust BW of pregnant and non-pregnant beef cows

Variable to be estimated	Predictors variables	Equation code	Relation
Non-pregnant cows			
SBW_{np}	BW	{4	$SBW = 0.8084 \times BW^{1.0303}$
EBW_{np}	SBW_{np}	{11 or {12	$EBW_{np} = 0.9092 \times SBW_{np}$, or $EBW_{np} = 0.8424 \times SBW_{np}^{1.0122}$
Pregnant cows			
SBW_p	BW	{4	$SBW = 0.8084 \times BW^{1.0303}$
SBW_{np}	SBW_p and PREG	-	$SBW_{np} = SBW_p - PREG$
PREG	If DOP ≤ 240: GU_{dp} If DOP > 240: GU_{dp} and UD_{dp}	-	If DOP ≤ 240: $PREG = GU_{dp}$ If DOP > 240: $PREG = GU_{dp} + UD_{dp}$
GU_{dp}	GU and UT_{np}	-	$GU_{dp} = GU - UT_{np}$
GU	DOP or DOP and BCS	{5 or {6	$GU = 0.2243 \times BCS^{0.3225} \times e^{((0.02544 - 0.0000286 \times DOP) \times DOP)}$, or $GU = 0.2106 \times e^{((0.03119 - 0.00004117 \times DOP) \times DOP)}$
UT_{np}	SBW_p and GU	{8	If DOP ≤ 240: $UT_{np} = 0.0012 \times (SBW_p - GU + 0.6)$ If DOP > 240: $UT_{np} = 0.0012 \times (SBW_p - GU + 0.6 - 2)$
UD_{dp}	UD_{np} and DOP	{10	$UD_{dp} = UD_{np} \times e^{((DOP - 238) \times 0.0109)} - UD_{np}$
UD_{np}	SBW_p and BCS	{9	$UD_{np} = SBW_{np} \times 0.00589 \times BCS^{0.2043}$, or If DOP ≤ 240: $UD_{np} = (SBW_p - GU_{dp}) \times 0.00589 \times BCS^{0.2043}$ If DOP > 240: $UD_{np} = (SBW_p - GU_{dp} - 2) \times 0.00589 \times BCS^{0.2043}$
EBW_p	EBW_{np} and PREG	-	$EBW_p = EBW_{np} + PREG$
EBW_{np}	SBW_{np}	{11 or {12	$EBW_{np} = 0.9092 \times SBW_{np}$, or $EBW_{np} = 0.8424 \times SBW_{np}^{1.0122}$

Figure 1

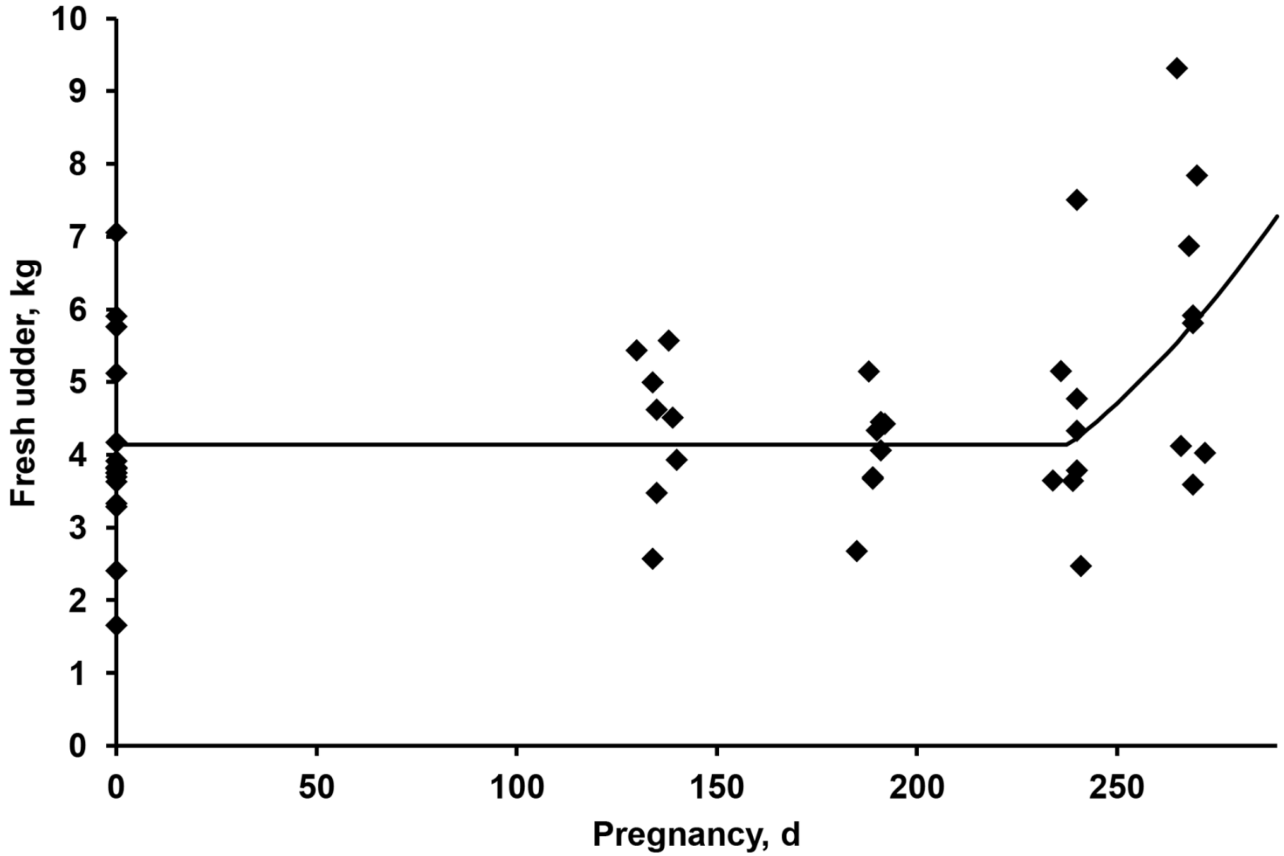
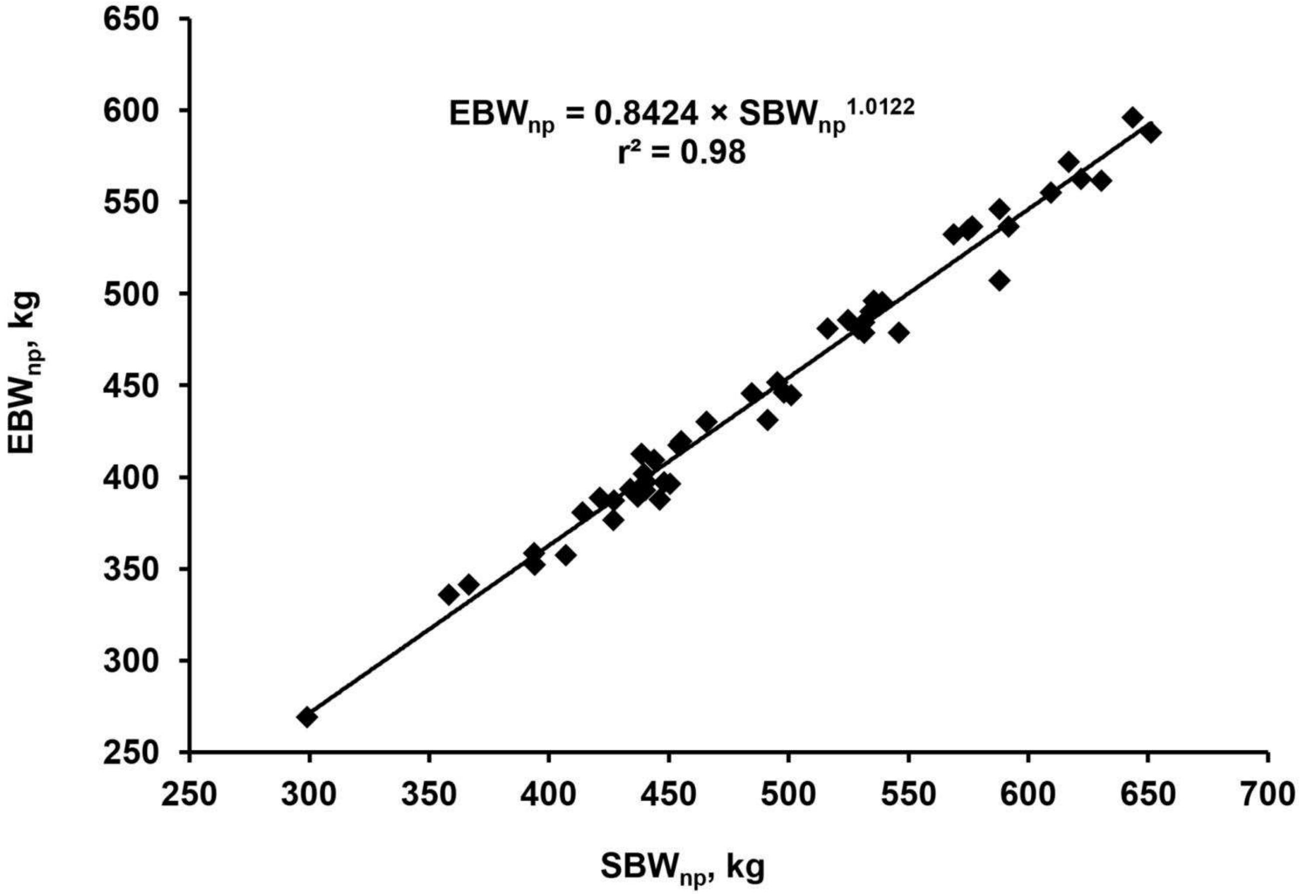


Figure 2



EBWnp = 0.8424 × SBWnp^1.0122
r² = 0.98
650
600
550
500
450
400
350
300
250
EBWnp, kg
250
300
350
400
450
500
550
600
650
700
SBWnp, kg

APPENDIX

For exemplification of practical application an example can be used:

A pregnant cow with initial BW of 500 kg, BCS = 5 and with 120 of pregnancy, is subjected to a supposed treatment during a 150 d period; and at the end of this period is with 600 kg of BW, BCS = 6 and DOP = 270 d. The objective is to know the gain related to the maternal tissues and the gain related to pregnancy.

Initial

BW = 500 kg
DOP = 120 d
BCS = 5
$SBW_p = 0.8084 \times 500^{1.0303} = 487.95$ kg
$GU = 0.2243 \times 5^{0.3225} \times e^{((0.02544 - 0.0000286 \times 120) \times 120)} = 5.29$ kg
$UT_{np} = 0.0012 \times (487.95 - 5.29 + 0.6) = 0.58$ kg
$GU_{dp} = 5.29 - 0.58 = 4.71$ kg
$UD_{np} = (487.95 - 5.29) \times 0.00589 \times 5^{0.2043} = 3.95$ kg
$UD_{dp} = 0$ kg

$PREG = GU_{dp} = 4.71$ kg

$SBW_{np} = 487.95 - 4.71 = 483.24$ kg
$EBW_{np} = 0.8424 \times 483.24^{1.0122} = 438.97$ kg
$EBW_p = 438.97 + 4.71 = 443.67$ kg

Final

BW = 600 kg
DOP = 270 d
BCS = 6
$SBW_p = 0.8084 \times 600^{1.0303} = 588.78$ kg
$GU = 0.2243 \times 6^{0.3225} \times e^{((0.02544 - 0.0000286 \times 270) \times 270)} = 47.80$ kg
$UT_{np} = 0.0012 \times (588.78 - 47.80 + 0.6 - 2) = 0.65$ kg
$GU_{dp} = 47.80 - 0.65 = 47.15$ kg
$UD_{np} = (588.78 - 5.29 - 2) \times 0.00589 \times 6^{0.2043} = 4.58$ kg
$UD_{dp} = 4.58 \times e^{((270 - 238) \times 0.0109)} - 4.58 = 1.90$ kg

$PREG = GU_{dp} + UD_{dp} = 47.15 + 1.90 = 49.05$ kg

$SBW_{np} = 588.78 - 49.05 = 539.74$ kg
$EBW_{np} = 0.8424 \times 539.74^{1.0122} = 490.94$ kg
$EBW_p = 490.94 + 49.05 = 539.99$ kg

Total BW gain = 600 – 500 = 100 kg
Total SBW gain = 588.78 – 487.95 = 100.83 kg
Total EBW gain = 539.99 – 443.67 = 96.32 kg

ADG = 100 / 150 = 0.667 kg/d
SBG = 100.83 / 150 = 0.672 kg/d
EBG = 96.32 / 150 = 0.642 kg/d

Partial portions of the gain

Total PREG gain = 49.05 – 4.71 = 44.34 kg (46% of total EBG)
Total maternal tissues EBW gain = 490.94 – 438.97 = 51.98 kg (54% of total EBG)

rSBG = (539.74 – 483.24) / 150 = 0.377 kg/d
rEBG = (490.94 – 443.67) / 150 = 0.347 kg/d

where DOP = days of pregnancy, BCS = body condition score, SBW_p = shrunk body weight of a cow in pregnant condition, GU = gravid uterus, UT_{np} = uterus plus ovaries of a cow in non-pregnant condition, GU_{dp} = accretion of gravid uterus due to pregnancy, UD_{np} = udder of a cow in non-pregnant condition, UD_{dp} = accretion of udder due to pregnancy, PREG = pregnant compound, SBW_{np} = shrunk body weight of a cow in non-pregnant condition, EBW_{np} = empty body weight of a cow in non-pregnant condition, EBW_p = empty body weight of a cow in pregnant condition, ADG = average daily gain, SBG = shrunk body gain, EBG = empty body gain, rSBG = real shrunk body gain (refers only to the gain of maternal tissues) and rEBG = real empty body gain (refers only to the gain of maternal tissues).

CHAPTER 3

Effect of pregnancy and feeding level on voluntary intake, digestion and microbial N production in Nellore cows

ABSTRACT: The objective of this experiment was to evaluate the effects of pregnancy and feeding level on intake, digestibility and efficiency of microbial N production in Nellore cows. Forty-four multiparous Nellore cows (32 pregnant and 12 non-pregnant) with average initial body weight of 451 ± 10 kg were fed either **HIGH** (*ad libitum*) or **LOW** (restricted feeding 1.2 times maintenance according to the NRC) feeding level. The diet consisted of corn silage (85%), ground corn, soybean meal, urea and mineral mixture. The intake was controlled daily and the DM intake (DMI) was evaluated weekly. In vivo apparent total digestibility was estimated using indigestible NDF as an internal marker and microbial N synthesis was estimated from the technique of the purine derivatives in urine. The voluntary feed intake reduced as the pregnancy advances in Nellore cows and can be calculated as DMI (kg/d) = (16 – 0.0093 × days in pregnancy) / 1000 × SBW_p or DMI (kg/d) = (16.4 – 0.0093 × days in pregnancy) / 1000 × SBW_{np}. The average DMI of LOW-fed cows corresponded to 102, 98 and 67% of the amount of energy necessary to attend the maintenance and pregnancy energy requirements suggested by NRC for a cow at 0, 135 and 270 d of pregnancy, respectively. LOW-fed cows had 0.26 kg/d of average shrunk body gain indicating that the nutrient energy requirements of Zebu cows are likely lower than those suggested by NRC. The interaction between the feeding level and days of pregnancy was significant ($P<0.05$) for the digestibility of DM, OM, N, EE, NDF_{ap} and GE, and the values of TDN. In all these cases there was a reduction in digestibility with increasing gestation age in HIGH-fed cows, while the digestibility OM, N, EE, NDF_{ap} and GE increased as function of days of pregnancy in LOW-fed. The reduction in the digestibility of neutral detergent fiber occurs faster than in dry matter digestibility. These data suggests that the reduction of the digestibility as pregnancy increases is caused by an increase in the rate of passage as compensation factor for the ruminal volume reduction. There were no direct effects of pregnancy on microbial N production in Nellore cows.

KEYWORDS: beef cattle, *Bos indicus*, dry matter intake, gestation, total digestible nutrients, Zebu

RESUMO: O objetivo deste estudo foi avaliar os efeitos da gestação e do nível alimentar sobre o consumo, a digestibilidade e a eficiência de produção microbiana em vacas Nelore. Quarenta e quatro vacas Nelore adultas, multíparas (32 gestantes e 12 não gestantes) com peso médio inicial de 451 ± 10 kg foram alimentadas a um nível alto (ad libitum) ou baixo (alimentação restrita a 1,2 vezes a mantença, de acordo com o NRC) da mesma dieta. A dieta consistiu de silagem de milho (85%), milho grão moído, farelo de soja, uréia e mistura mineral. O consumo foi controlado diariamente e o consumo diário de matéria seca (CMS) foi avaliado semanalmente. A digestibilidade aparente in vivo foi estimada usando-se FDNi como marcador interno da digesta. A produção de N microbiano foi avaliada pela técnica dos derivados de purina na urina. O consumo voluntário reduziu com o aumento da gestação e pode ser descrito como CMS (kg/d) = (16 – 0,0093 × dias de gestação) / 1000 × PCVZ ou CMS (kg/d) = (16,4 – 0,0093 × dias de gestação) / 1000 × $PCVZ_{np}$. O CMS médio das vacas do baixo nível alimentar correspondeu a 102, 98 e 67% da quantidade de consumo necessário para atender as exigências de energia sugeridos pelo NRC para uma vaca aos 0, 135 e 270 dias de gestação, respectivamente. Vacas do baixo nível alimentar tiveram ganho médio diário de 0,26 kg, indicando que as exigências de energia estimadas pelo NRC são superestimadas para vacas zebuínas. A interação entre o nível alimentar e o tempo de gestação foi significativa (P<0,05) par as digestibilidades da matéria seca, matéria orgânica, nitrogênio, extrato etéreo, fibra em detergente neutro e energia bruta, e também para os valores de NDT. Em todos os casos, houve redução da digestibilidade em função do aumento da gestação nas vacas alimentadas ad libitum, enquanto que nas vacas que sofreram restrição alimentar as digestibilidades de matéria orgânica, nitrogênio, extrato etéreo e fibra em detergente neutro aumentaram em função do tempo de gestação. A redução da digestibilidade da fibra em detergente neutro em função do tempo de gestação ocorreu mais rapidamente do que da digestibilidade da matéria seca. Os dados desse estudo sugerem que a redução na digestibilidade ocorrida em função do tempo de gestação nas vacas alimentadas ad libitum tenha sido causada pelo aumento da taxa de passagem, como fator compensatório para a redução do volume ruminal. Não foram observados efeitos da gestação sobre a produção de N microbiano em vacas Nelore.

Palavras-chave: *Bos indicus*, consumo de matéria seca, gestação, nutrientes digestíveis totais, Zebu

INTRODUCTION

Pregnancy is the most complex physiological stage of a beef cow because several modifications occur in the normal physiology status of the cow to support the formation of a new individual of the species. These modifications may also affect the feed intake and the dietary utilization of feeds.

Simple stomach mammals increase feed intake during pregnancy to coincide with the high nutritional requirements of large litters of simple fetuses (Forbes, 2007). However, in ruminant animals, stomachs occupy a greater proportion of the abdominal cavity and are more subject to compression effect than in non-ruminants. It is suggested that cows may increase the voluntary feed intake in the middle gestation (Ingvartsen et al., 1992), nonetheless there is noticeable reduction in feed intake in the final weeks of pregnancy (Ingvartsen and Andersen, 2000).

Studies evaluating the effects of pregnancy on digestibility in cattle are limited (Ivins, 1960; Lamberth, 1969; Hanks et al., 1993). The relationship between feed intake and digestibility in ruminants is well known (Conrad et al., 1964; Colucci et al., 1982; Edionwe and Owen, 1989) but the effects of pregnancy on this relationship remains unclear, noticeably when considering *B. indicus* cattle.

The objective of this experiment was to evaluate the effects of pregnancy and feeding level on intake, digestibility and production of microbial N in the rumen of Nellore cows.

MATERIALS AND METHODS

Animals

This study was conducted at the Federal University of Viçosa (Viçosa, MG, Brazil), following the standard procedures for humane animal care and handling according to the guidelines of Federal University of Viçosa (Brazil).

Forty-four multiparous Nellore cows with average initial body weight of 451 ± 10 kg, age of 5.6 ± 0.5 years and body condition score of 4.4 ± 0.2 (1 to 9 scale) were used in this study. Animals and reproductive management prior to the beginning of the experiment has been already reported by by Gionbelli (2013). From the initial 44 cows, 32 were separated at random and hand mated with Nellore bulls to form the pregnant group and the remained 12 cows were assigned to the non-pregnant group.

Diet and management

Cows were housed in pens (48 m², 5-6 cows per pen) with a concrete floor, 15 m² of covered area, and *ad libitum* access to fresh water. Feed intake was measured individually using an electronic head gate system (Kloppen Soluções Tecnológicas, Pirassununga, SP, Brazil). Pregnant cows were at 47 ± 3 d of gestation when the feeding trial started.

All cows were fed the same diet twice daily (0700 and 1500 h) and segregated into 2 groups according to level of feeding: **HIGH** (*ad libitum*) or **LOW** (restricted feeding 1.2 times maintenance according to the NRC (restricted feeding 1.2 times maintenance according to the NRC 2000). Sixteen pregnant and 5 non-pregnant cows were fed HIGH level and 16 pregnant and 7 non-pregnant cows were LOW-fed. The restricted feeding was estimated so that 1.2 times maintenance sustained pregnancy and the HIGH-fed allowed some maternal tissue storage. The cows had ad libitum access to water.

The diet consisted of corn silage, ground corn, soybean meal, urea and mineral mixture (Table 1). The feed supplied and the orts were recorded daily. Samples of corn silage and orts were taken daily and every week a composite sample was collected for analyses. Samples of the ground corn and soybean meal were taken each time the concentrate portion was mixed.

The cows used in this experiment were from a comparative slaughter experiment in which part of cows was harvested throughout the experiment. The 32 pregnant cows were separated at random into 4 groups of 8 cows each (4 cows per each feeding level) and harvested at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. As such, the evaluations were performed using 32 pregnant cows (16 of HIGH and 16 of LOW feeding level) up to 136 d, 24 up to 189 d (12 HIGH and 12 LOW-fed), 16 up to 239 d (8 HIGH and 8 LOW-fed) and 8 up to 269 d of gestation (4 HIGH and 4 LOW-fed). The non-pregnant cows were harvested at different times of the experiment (85 to 216 days of feeding control) in order to keep them in experiment for a similar amount of time as the pregnant cows.

Dry matter intake

The amounts of provided feed and orts were measured and sampled daily, stored at -20ºC and weekly pooled for analyses. Thus, the data of dry matter intake for each cow was obtained weekly. The dry matter intake in proportion of BW (g/kg) for each cow was obtained at each period of fecal sampling, when cows were weighed. The values of shrunk BW (**SBW**) e SBW in pregnant (**SBW_p**) and non-pregnant condition (**SBW_{np}**) were calculated according Gionbelli (2013).

Digestibility

In vivo apparent total digestibility was estimated using indigestible NDF (iNDF) as internal marker. Fecal samples were collected from each cow during 5 consecutive d (at 1800, 1500, 1200, 0900 and 0600 h for the first to fifth d, respectively) in 9 collection periods. The interval between collection periods varied from 21 to 28 d. The intervals were irregulars to permit a maximum number of collections for each cow before harvest. Fecal samples (60 g of wet weight of each d of collection) were pooled for each animal in each period and stored at -20ºC for further analyses. Total tract DM, OM, N, EE, NDF corrected for ash and protein (**NDF_{ap}**), NFC and GE apparent digestibilities were estimated as the coefficient of the total tract disappearance (intake minus excretion) and intake.

Microbial N production

Microbial N synthesis was estimated by using the technique of the purine derivatives in urine (Chen and Gomes, 1992). The spot sampling was used to assess the excretion of urinary nitrogenous compounds (Valadares et al., 1999). Two spot samples of urine were collected in each collection period at the second and the fifth d of fecal sampling, at 1600 and 0800 h, respectively, as proposed by Pereira (2009). Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine excretion (mg/d) of $37.88 \times SBW^{0.9316}$ (Santos et al., unpublished data). The urinary concentrations of creatinine, allantoin and uric acid were obtained according to George et al. (2006).

Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions, which were obtained by the product between their concentrations in urine by the daily urinary volume. Absorbed purines were calculated from the excretion of purine derivatives (Prates et al., 2012) as follows:

$$Y = \frac{X - 0.405 \times BW^{0.75}}{0.99} \qquad \text{Eq. [1]}$$

where Y = absorbed purines (mmol/d), X = excretion of purine derivates (mmol/d), 0.99 = recovered absorbed purines. The 0.405 × $BW^{0.75}$ value = endogenous excretion of purine derivates. Ruminal synthesis of nitrogen compounds was calculated as a function of the absorbed purines (Prates et al., 2012) as follows:

$$Y = \frac{70 \times X}{0.93 \times 0.11 \times 1000} \qquad \text{Eq. [2]}$$

where Y = ruminal synthesis of nitrogen compounds (N/d, g), X = absorbed purines (mmol/day), 70 = purine N content (mgN/mol), 0.93 = purine digestibility and 0.11 = relation of purine N:total N of microorganisms.

Chemical analyses

All samples were lyophilized. Portions of 20 g of the samples were ground using a Willey mill (TE-650, Tecnal, Piracicaba, SP, Brazil) to pass a 1 mm screen for analyses of DM, ash, N, EE and NDF. Another portion of 20 g of each sample was ground to pass a 2 mm screen for iNDF analisys (Casali et al., 2008). The DM was collected by oven drying at 100 ºC for 18 hours (Method 934.01; AOAC, 2000). The ash content was determined by 2-h incineration process in a 600 ºC muffle furnace (Method 942.05; AOAC, 2000). The CP content was calculated based on the N content multiplied by the 6.25 factor. The N content was calculated by Kjeldahl procedure (Method 920.87; AOAC, 2000). Evaluation of the EE content was performed following the AOAC method (Method 920.39; AOAC, 2000) using a Soxtherm 2000 extractor (Gerhardt, Bonn, Germany) using petroleum ether similar to the procedure of Palmquist and Jenkins (2003). The content of NDF was obtained according to method described by Mertens (2002), with addition of sodium sulfite (Undersander et al., 1993) for analyses of soybean meal, because of the gelatinization of the protein content. Ankom 200 system (Ankom, Macedon, NY) was used for the NDF evaluations, with heat-stable α-amylase addition and modification of the bag utilized (5.0 × 5.0 cm, Casali et al., 2009), which was manufactured using nonwoven textile (100 g/m^2). In all the samples NDF content was corrected for ash and protein. Non-fibrous carbohydrates were calculated as suggested by Detmann and Valadares Filho (2010), where NFC = 100 – [(%CP - %CP derived from urea + %urea) + NDF_{ap} + EE + ash], where NDF_{ap} = neutral detergent fiber corrected for ash and protein. Gross energy was measured using an adiabatic bomb calorimeter (C5001, IKA-Werke Co, Staufen, Germany).

For iNDF analysis the samples were incubated in situ for 10 d (Casali et al., 2008) in the rumen of 3 Holstein × Nellore cows that had ad libitum access to the same diet used in the experiment. Residual samples were analyzed for the NDF concentration as previously described.

Statistical Analysis

The procedure MIXED of SAS version 9.2 (SAS Inst. Inc., Cary, NC) was used to evaluate the effects of feeding level and pregnancy on the studied variables, considering also the effect of the collection periods, the repeated measures in the same animal, and also the possible interactions between the mentioned factors. A previous evaluation of the estimated TDN of the diet based in the chemical composition (Detmann et al., 2010) revealed a significant variation between collection periods justifying the use of period of measure as random effect in the model.

The response variables were evaluated as follows:

$$Y_{ijk} = \mu + F_k + \beta_1(G_l) + \beta_2(F\times G)_{kl} + \delta_{kl} + \varepsilon_{ijkl}$$

where:

μ = the overall mean

β_1 and β_2 = the regression coefficients

F_k = the fixed effect of feeding level

G_l = the fixed effect of days of gestation

$(F\times G)_{kl}$ = the interaction between feeding level and days of gestation

δ_{kl} = the random error with mean 0 and variance σ^2_δ. The variance between cows (subjects) within feeding level and days of gestation and it is equal to the covariance between repeated measurements within cows

ε_{ijkl} = random error with the mean 0 and variance σ^2, the variance between measurements within cows

Because the interval between collections periods varied the response variables are considered to be measured irregularly. Under these circumstances a continuous-time model was used to describe the covariances among the errors as proposed by Moser (Moser, 2004). The spatial data covariance structures provided in PROC MIXED of SAS was used. The spatial power was used as covariance function.

Least square means were estimated for feeding level. The value of 0.05 was adopted as critical level of probability for occurrence of Type I error.

RESULTS

As result of the feeding levels applied, the final SBW was different (P=0.003) among feeding level groups with average of 564 ± 6 kg for HIGH-fed cows and 481 ± 5 kg for LOW-fed cows. This was due to the difference of shrunk body weight daily gain (**SBG**, $P<0.001$) among feeding level groups which was 0.86 ± 0.04 kg/d for HIGH-fed cows and 0.26 ± 0.04 kg/d for LOW-fed cows. Additionally, comparisons within each period of gestation evaluated showed that HIGH-fed cows had greater ($P<0.001$) SBG than LOW-fed cows at all gestational periods evaluated. The complete results of weight gain, body composition, and weight variation of body components and organs of cows used in this study were presented by Gionbelli (2013).

Dry matter intake

A graphical representation of individual dry matter intake data of pregnant cows is shown in Figure 1. Because the level of intake of LOW-fed cows was established the interaction between feeding level and days of gestation should be significant so that the effect of gestational age would significant. The interaction between feeding level and days of pregnancy on the DMI presented as SBW proportion was significant ($P\leq0.018$, Table 2). The DMI reduced as proportion of SBW as a function of days of pregnancy. To eliminate probable misinterpretation effects and to certify the true relationship between the voluntary intake and days of gestation a new analysis was performed removing the LOW-fed cow's information from the database and the feeding level from the statistic model to check also the possible quadratic and cubic effects on DMI. Only the linear parameter was significant (P=0.012, 0.522 and 0.739, respectively). Thus, the DMI in Nellore cows fed ad libitum can be described as follows (based on functions showed in Table 2):

DMI (kg/d) = $(16 - 0.0093 \times DP) / 1000 \times SBW_p$ or,

DMI (kg/d) = $(16.4 - 0.0093 \times DP) / 1000 \times SBW_{np}$

Digestibility

Except for the NFC digestibility, the interaction between feeding level and days of pregnancy was significant ($P<0.05$) for all the other constituents (Table 3). Except for DM digestibility and TDN content there was a reduction in digestibility with increasing gestation age in HIGH-fed cows, while the digestibility of LOW-fed increased with the increase in days of pregnancy. The DM, OM, NDF_{ap}, and GE digestibility coefficients were higher in LOW-fed cows ($P<0.05$). The EE digestibility was higher in HIGH-fed cows than in LOW-fed cows ($P=0.015$).

Except for EE and NFC, the digestibilities of the other components of the diet as well as the contents of TDN were affected by the pregnancy similarly to DM digestibility in HIGH-fed cows. The relationship between the DM digestibility and gestational age is graphically presented in Figure 2 including the functions of Table 3.

Microbial N production

There were no effects of days of pregnancy ($P\geq0.180$) and interaction between feeding level and pregnancy ($P\geq0.368$) on microbial N production and efficiency (Table 3). The microbial N production (g/d) was higher in HIGH-fed cows than in LOW-fed cows ($P<0.001$). However, the efficiency of microbial N production by g of N intake, kg of digestible organic matter intake and total digestible nutrients was higher in LOW-fed cows ($P=0.021$, 0.051 and 0.041) than in HIGH-fed cows, probably due to higher retention time and lower passage rate in restricted cows, allowing greater microbial fermentation of feed.

DISCUSSION

Dry matter intake

The voluntary DMI by BW unit observed for pregnant and non-pregnant cows (Table 2) was lower than usually observed for growing and lactation Zebu animals (Detmann et al., 2003). Using the data of the current study the energy requirements of a 510 kg BW cow (average of the current study) for maintenance and pregnancy at 135 or 270 d were estimated according to NRC (2000). When the energy requirements were contrasted to the energy intake from the current diet, the observed energy intake of LOW-fed cows was equal to 102, 98 and 67% of the estimated energy requirements according to the NRC (2000) at 0, 135 and 270 d of pregnancy, respectively. In HIGH-fed cows, the observed energy intake was equal to 168, 162 and 111% of the energy requirements at the same times estimated for the previous group. In the current study LOW-fed cows had a SBG of 0.26 kg/d (pregnant and non-pregnant) indicating that the energy requirements of Zebu cows are probably lower than those suggested by NRC (2000) based on *B. taurus* cows.

Previous studies suggest that the voluntary DMI in pregnant cows reduces in late gestation (Lamberth, 1969; Ingvartsen et al., 1992; Forbes, 1996; Ingvartsen and Andersen, 2000). Ingvartsen et al. (1992) compiled data from 20 groups of pregnant cows from 9 studies and observed variations on DMI in late gestation ranging from increase of 0.2%/week to decrease of 9.4%/week. The same authors also reported that pregnant heifers reduce the voluntary DMI in the last 14 weeks of gestation at a rate of 1.53%/week, and in about 30% in the last five days before parturition.

A reduction in the weight of rumen-reticulum and omasum due to increase in gestation time was observed by Gionbelli (2013) using the same cows of the current study. The authors also reported that the reduction of 8.18% observed on the weight of the rumen between 136 and 239 d of gestation is a reduction of approximately 37% in volume (80.0 to 58.3 liters). This reduction is possibly caused by limited space. It has been suggested that the compression of the rumen by gravid uterus and visceral fat reduce the dry matter intake in late gestation in ruminant animals (Forbes, 2007). Forbes (1968) observed drastic reduction in ruminal volume when ewes were slaughtered and immediately frozen and then sawn in cross-sections, at various stages of gestation. Although there has been a reduction in reticulum-rumen capacity, is important to note that there is also increase of paunch girth to try reduce the effect of ruminal compression. Bereskin and Touchberry (1967) reported that there is an increase of up to 5 per cent in paunch girth of cows in late pregnancy.

The regulation of DMI in pregnant cows may present physical and physiological factors which are not included in traditional patterns of regulation of feed intake in ruminants (Fisher et al., 1987). These peculiarities, such as the influence of calf weight on reduction of reticulum-rumen volume, the hormonal regulation of pregnancy, or the homeorhetic mechanism of nutrient use, are difficult to model and are the main causes of changes in voluntary intake observed in this physiological stage of cattle.

Digestibility

The HIGH-fed cows reduced the DM digestibility in function of gestational age by one point percent at each 107 days (1/0.0093), corresponding to 2.7 percentile points of difference between a non-pregnant and a pregnant cow at parturition (aprox. 290 days of gestation for Nellore cows; Cavalcante et al., 2001; Rocha et al., 2005). Ivins (1960) suggested that digestibility coefficients are determined largely by the feed, whereas other factors such as physiological states vary digestibility by up to six units only.

It is suggested that the reduction of the digestibility as days of pregnancy increased in HIGH-fed cows was possibly caused by an increase in the passage rate as compensation factor for the ruminal volume reduction. Previous studies showed that the passage rate is higher in the late gestation in cattle (Hanks et al., 1993) and sheep (Coffey et al., 1989; Gunter et al., 1990; Kaske and Groth, 1997). The greater reduction in NDF_{ap} digestibility (Table 3) is in agreement with this hypothesis. The NDF_{ap} digestibility reduce by one point percent each 62 days (1/0.0161), that was almost twice than in DM digestibility. In a study with cannulated pregnant and non-pregnant beef cows Hanks et al. (1993) observed lower gastrointestinal retention times, ruminal retention times, intestinal transit time and gastrointestinal fill for pregnant than non-pregnant cows. On the other hand, the increase in the digestibility of LOW-fed cows may be an effect of increase in efficiency of restricted cows with the increase in pregnancy and the long time receiving low amounts of feed.

Microbial N production

Complex factors are involved with the ruminal microbial N production along with the complication that there are great difficulties to measure it in a proper way (Dewhurst et al., 2000). The availability and synchronization between energy and N in the rumen have been recognized as the most important factors affecting microbial protein synthesis (Russell et al., 1992).

Studies evaluating the direct effects of pregnancy on microbial N production were not found in the literature. The variations in microbial N production during pregnancy are probably related to effects of the feed. It is suggested that the greater microbial N efficiency in LOW-fed cows is due to the higher ruminal retention time (Cherney et al., 1991).

In conditions where the passage rate is high, it is expected reduction in microbial maintenance costs due to a reduction in the ruminal retention time. The passage rate is function of level of dry matter intake. Thus, is usually assumed that the efficiency of rumen microbial synthesis can be increased by increasing dry matter intake (Verbic, 2002). However, this assumption is mostly correct when the passage rate is increased or decreased as a function of feed quality. In another words, when there is a positive relationship between passage rate and digestibility of the feed. When the passage rate is decreased by feed restriction, as in this study, it is suggested that the higher ruminal retention time allows greater ruminal degradation of the feed and consequently greater microbial N production by feed unit (Table 3).

CONCLUSION

The voluntary feed intake reduces as the pregnancy age increase in Nellore cows. On the other hand the digestibility reduces linearly as the gestation increases in the cows fed ad libitum. There are no direct effects of pregnancy on microbial N production in Nellore cows.

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REFERENCES

AOAC, 2000. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, Arlington, VA.

Bereskin, B., Touchberry, R.W., 1967. Some Effects of Pregnancy on Body Weight and Paunch Girth1. J. Dairy. Sci. 50, 220-224.

Casali, A.O., Detmann, E., Valadares Filho, S.C., Pereira, J.C., Cunha, M., Detmann, K.S.C., Paulino, M.F., 2009. Estimação de teores de componentes fibrosos em alimentos para ruminantes em sacos de diferentes tecidos. Revista Brasileira de Zootecnia 38, 130-138.

Casali, A.O., Detmann, E., Valadares Filho, S.C., Pereira, J.C., Henriques, L.T., Freitas, S.G., Paulino, M.F., 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. Revista Brasileira de Zootecnia 37, 335-342.

Cavalcante, F.A., Martins Filho, R., Campello, C.C., Lobo, R.N.B., Martins, G.A., 2001. Período de gestação em rebanho Nelore na Amazônia Oriental. Revista Brasileira de Zootecnia 30, 1451-1455.

Chen, X.B., Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of the technical details. Rowett Research Institute, Bucksburnd Aberdeen.

Cherney, D.J., Mertens, D.R., Moore, J.E., 1991. Fluid and particulate retention times in sheep as influenced by intake level and forage morphological composition. J. Anim. Sci. 69, 413-422.

Coffey, K.P., Paterson, J.A., Saul, C.S., Coffey, L.S., Turner, K.E., Bowman, J.G., 1989. The Influence of Pregnancy and Source of Supplemental Protein on Intake, Digestive Kinetics and Amino Acid Absorption by Ewes. J. Anim. Sci. 67, 1805-1814.

Colucci, P.E., Chase, L.E., Van Soest, P.J., 1982. Feed Intake, Apparent Diet Digestibility, and Rate of Particulate Passage in Dairy Cattle. J. Dairy. Sci. 65, 1445-1456.

Conrad, H.R., Pratt, A.D., Hibbs, J.W., 1964. Regulation of Feed Intake in Dairy Cows. I. Change in Importance of Physical and Physiological Factors with Increasing Digestibility1. J. Dairy. Sci. 47, 54-62.

Detmann, E., Queiroz, A.C.d., Cecon, P.R., Zervoudakis, J.T., Paulino, M.F., Valadares Filho, S.d.C., Cabral, L.d.S., Lana, R.d.P., 2003. Consumo de fibra em detergente neutro por bovinos em confinamento. Revista Brasileira de Zootecnia 32, 1763-1777.

Detmann, E., Valadares Filho, S.C., 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arq. Bras. Med. Vet. Zootec. 62, 980-984.

Detmann, E., Valadares Filho, S.C., Paulino, M.F., 2010. PREDICTION OF THE ENERGY VALUE OF CATTLE DIETS BASED ON THE CHEMICAL COMPOSITION OF FEEDS. In: Valadares Filho, S.C., Marcondes, M.I., Chizzotti, M.L., Paulino, P.V. (Eds.), Nutrient Requirements of Zebu Beef Cattle - BR-CORTE. Suprema Gráfica e Editora, Viçosa, pp. 45-60.

Dewhurst, R.J., Davies, D.R., Merry, R.J., 2000. Microbial protein supply from the rumen. Animal Feed Science and Technology 85, 1-21.

Edionwe, A.O., Owen, F.G., 1989. Relation of Intake to Digestibility of Diets Containing Soyhulls and Distillers Dried Grains1. J. Dairy. Sci. 72, 1786-1792.

Fisher, D.S., Burns, J.C., Pond, K.R., 1987. Modeling ad libitum dry matter intake by ruminants as regulated by distension and chemostatic feedbacks. Journal of Theoretical Biology 126, 407-418.

Forbes, J.M., 1968. The physical relationships of the abdominal organs in the pregnant ewe. The Journal of Agricultural Science 70, 171-177.

Forbes, J.M., 1996. Integration of regulatory signals controlling forage intake in ruminants. J. Anim. Sci. 74, 3029-3035.

Forbes, J.M., 2007. Voluntary Food Intake and Diet Selection in Farm Animals. CABI Publishing, Wallingford, UK.

George, S.K., Dipu, M.T., Mehra, U.R., Singh, P., Verma, A.K., Ramgoakar, J.S., 2006. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. Journal of Chromatography B 832, 134-137.

Gionbelli, M.P., 2013. NUTRIENT REQUIREMENTS AND QUANTITATIVE ASPECTS OF GROWTH, DEVELOPMENT AND DIGESTION OF PREGNANT AND NON-PREGNANT NELLORE COWS. Departamento de Zootecnia. Universidade Federal de Viçosa, Viçosa, p. 198.

Gunter, S.A., Judkins, M.B., Krysl, L.J., Broesder, J.T., Barton, R.K., Rueda, B.R., Hallford, D.M., Holcombe, D.W., 1990. Digesta kinetics, ruminal fermentation characteristics and serum metabolites of pregnant and lactating ewes fed chopped alfalfa hay. J. Anim. Sci. 68, 3821-3831.

Hanks, D.R., Judkins, M.B., McCracken, B.A., Holcombe, D.W., Krysl, L.J., Park, K.K., 1993. Effects of pregnancy on digesta kinetics and ruminal fermentation in beef cows. J. Anim. Sci. 71, 2809-2814.

Ingvartsen, K.L., Andersen, B.B., 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy. Sci. 83, 1573-1597.

Ingvartsen, K.L., Andersen, H.R., Foldager, J., 1992. Effect of Sex and Pregnancy on Feed Intake Capacity of Growing Cattle. Acta Agriculturae Scandinavica, Section A - Animal Science 42, 40-46.

Ivins, J.D., 1960. Digestibility data and grassland evaluation. Proceedings of the Eighth International Grassland Congress, Reading, p. 459.

Kaske, M., Groth, A., 1997. Changes in factors affecting the rate of digesta passage during pregnancy and lactation in sheep fed on hay. Reprod. Nutr. Dev. 37, 573-588.

Lamberth, J., 1969. The effect of pregnancy in heifers on voluntary intake, total rumen contents, digestibility and rate of passage. Austr. J. Exp. Agric. 9, 493-496.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. Journal of AOAC International 85, 1217-1240.

Moser, E.B., 2004. Repeated measures modeling with PROC MIXED. Proceedings of the 29th Annual SAS Users Group International (SUGI) Conference. SAS Institute, Montréal, CA, pp. 1-19 (Paper 188).

NRC, 2000. Nutrient Requirements of Beef Cattle. National Academy Press, Washington, DC.

Palmquist, D.L., Jenkins, T.G., 2003. Challenges with fats and fatty acid methods. J. Anim. Sci. 81, 3250-3254.

Pereira, V.S.A., 2009. Influência do peso corporal e das características de carcaça sobre a excreção de creatinina e utilização de coleta spot de urina para estimar a excreção de derivados de purinas e de compostos nitrogenados em novilhas Nelore. Programa de Pós-Graduação em Medicina Veterinária. Universidade Federal de Viçosa, Viçosa, MG, p. 55.

Prates, L.L., Valadares, R.F.D., Valadares Filho, S.C., Detmann, E., Santos, S.A., Braga, J.M.S., Pellizzoni, S.G., Barbosa, K.S., 2012. Endogenous fraction and urinary recovery of purine derivatives in Nellore and Holstein heifers with abomasal purine infusion. Livestock Science 150, 179-186.

Rocha, J.C.M.C., Tonhati, H., Alencar, M.M., Lôbo, R.B., 2005. Genetic parameters estimates for gestation length in beef cattle. Arq. Bras. Med. Vet. Zootec. 57, 784-791.

Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J., Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70, 3551-3561.

Undersander, D.J., Mertens, D.R., Thiex, N., 1993. Forage analyses procedures. In: Association, N.F.T. (Ed.). National Forage Testing Association, Omaha, p. 135.

Valadares, R.F., Broderick, G.A., Valadares Filho, S.C., Clayton, M.K., 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J Dairy Sci 82, 2686-2696.

Verbic, J., 2002. Factors affecting microbial protein synthesis in the rumen with emphasis on diets containing forages. Viehwirtschaftliche Fachtagung 29, 1-6.

Figure captions

Figure 1. Relationship between dry matter intake and days of gestation. Data of sixteen cows used in this study. The dots represent the weeks of evaluation

Figure 2. Relationship between dry matter digestibility and days of gestation. Full dots and continuous line represents HIGH-fed cows. Empty dots and dashed line represents LOW-fed cows. The lines fitted to data represent the functions showed in Table 2.

Table 1. Ingredients and chemical composition of the diet

Item	Silage	Concentrate	Diet
Ingredient , % of DM			
Corn Silage	100.0	-	84.3
Ground corn	-	54.6	8.5
Soybean meal	-	33.0	5.1
Urea	-	7.3	1.2
Sodium chloride	-	2.1	0.37
Ammonium sulfate	-	1.5	0.25
Dicalcium phosphate	-	1.3	0.23
Microminerals mixture[1]	-	0.17	0.028
Analyzed composition[2], %			
DM	28.0	89.2	37.6
OM	94.7	92.8	94.4
CP	7.8	44.1	13.5
EE	2.9	2.5	2.8
NDF_{ap}	45.8	8.2	39.9
iNDF	20.8	0.65	17.6
NDIN	38.2	7.0	11.4
NFC	38.2	53.0	40.6
TDN	-	-	66.6
GE (Mcal/kg)	3.82	3.49	3.77

[1]Zinc sulfate (56.3 %), manganese sulfate (26.2 %), copper sulfate (16.8 %), potassium iodate (0.37 %), cobalt sulfate (0.23 %) and sodium selenite (0.10 %).
[2]NDF_{ap} = neutral detergent fiber corrected to ash and protein, iNDF = indigestible neutral detergent fiber and NFC = non fibrous carbohydrates.

Table 2. Effects of feeding level and pregnancy on dry matter intake in Nellore cows

Variable		Functions	Feeding level		P-value[1]		
			Low	High	FL	DP	FL×DP
DMI, kg		-	4.60 ± 0.16	7.62 ± 0.16	<0.001	0.779	0.286
DMI, $g/kgSBW_p$	Low	$\hat{y} = 10.1 \pm 0.3$	10.1 ± 0.3	15.0 ± 0.3	<0.001	0.113	0.002
	High	$\hat{y} = 16.0 \pm 0.6 - 0.0093 \pm 0.003 \times DP$					
DMI, $g/kgSBW_{np}$	Low	$\hat{y} = 10.2 \pm 0.4$	10.2 ± 0.4	15.3 ± 0.4	<0.001	0.168	0.018
	High	$\hat{y} = 16.4 \pm 0.7 - 0.0109 \pm 0.004 \times DP$					

[1]FL= feeding level, DP = days of pregnancy and FL×DP = interaction between feeding level and days of pregnancy

Table 3. Effects of feeding level and pregnancy on digestibility and microbial N production in Nellore cows

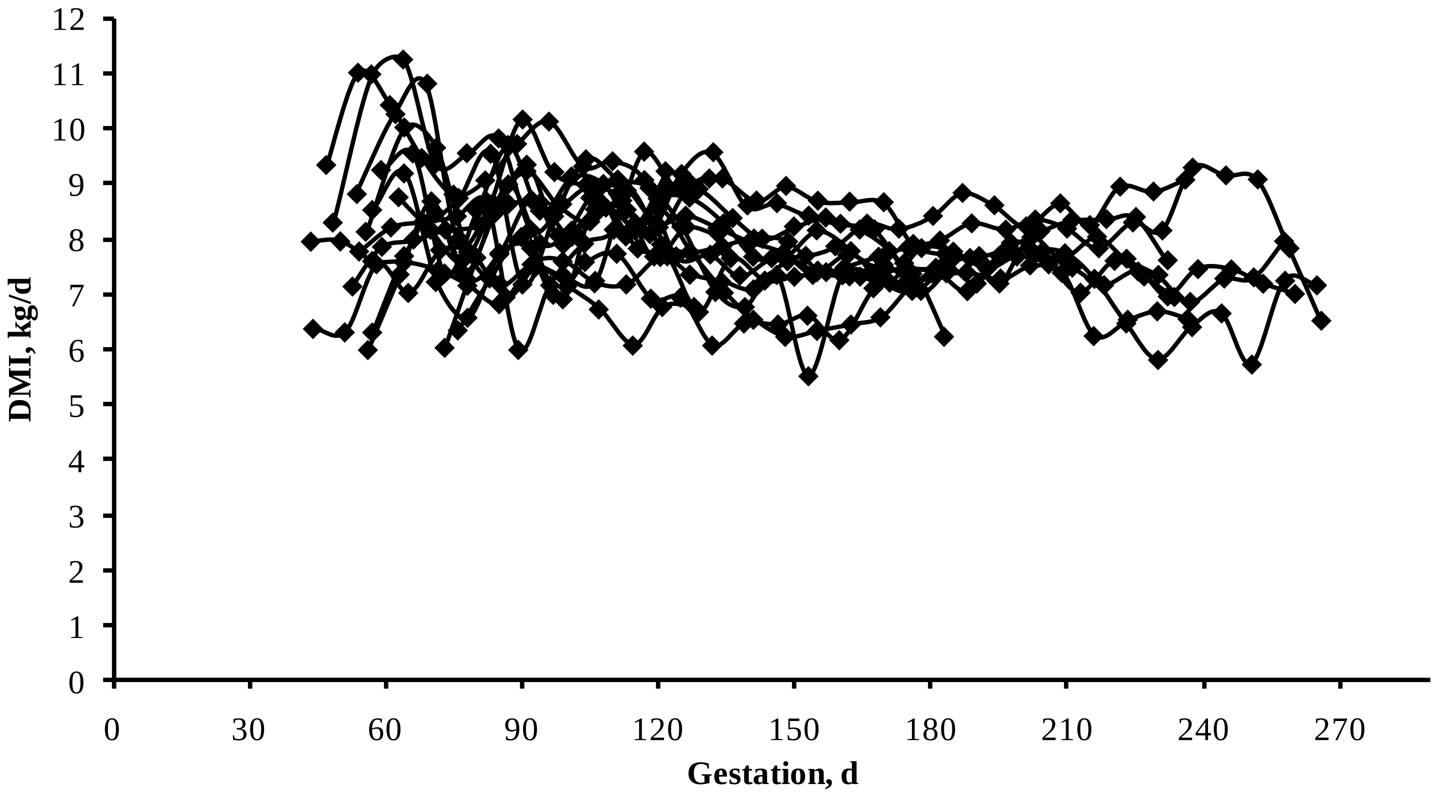
Variable[1]		Functions	Feeding level		P-value[2]		
			Low	High	FL	DP	FL×DP
Digestibility							
DM, %	Low[3] High	$\hat{y} = 62.8 \pm 1.6$ $\hat{y} = 60.9 \pm 1.6 – 0.0093 \pm 0.005 \times DP$	62.8 ± 1.6	59.9 ± 1.6	0.047	0.112	0.002
OM, %	Low[3] High	$\hat{y} = 65.5 \pm 1.6 + 0.0062 \pm 0.004 \times DP$ $\hat{y} = 64.0 \pm 1.6 – 0.0102 \pm 0.005 \times DP$	66.2 ± 1.4	62.9 ± 1.4	0.038	0.042	0.003
Nitrogenous compounds, %	Low High	$\hat{y} = 66.3 \pm 1.6 + 0.0083 \pm 0.004 \times DP$ $\hat{y} = 66.3 \pm 1.6 – 0.0053 \pm 0.005 \times DP$	67.1 ± 1.5	65.7 ± 1.6	0.217	0.722	0.011
EE, %	Low High	$\hat{y} = 80.8 \pm 1.2 + 0.0043 \pm 0.006 \times DP$ $\hat{y} = 83.2 \pm 1.2 – 0.0107 \pm 0.006 \times DP$	82.2 ± 0.9	82.1 ± 0.9	0.015	0.101	0.044
NDF_{ap}, %	Low High	$\hat{y} = 51.0 \pm 1.7 + 0.0052 \pm 0.007 \times DP$ $\hat{y} = 46.3 \pm 1.7 – 0.0161 \pm 0.008 \times DP$	51.5 ± 1.4	44.6 ± 1.4	0.045	<0.001	0.019
NFC, %	-	-	80.9 ± 1.6	80.0 ± 1.6	0.552	0.972	0.083
GE, %	Low High	$\hat{y} = 63.1 \pm 1.6 + 0.0069 \pm 0.004 \times DP$ $\hat{y} = 61.5 \pm 1.6 – 0.0107 \pm 0.005 \times DP$	63.8 ± 1.5	60.3 ± 1.5	0.035	0.032	0.002
TDN, %	Low[3] High	$\hat{y} = 67.6 \pm 1.4$ $\hat{y} = 66.0 \pm 1.6 – 0.0103 \pm 0.005 \times DP$	67.6 ± 1.4	64.9 ± 1.4	0.031	0.160	0.002
Microbial N production							
Nmic, g/d		-	49.6 ± 3.1	74.4 ± 3.2	<0.001	0.744	0.617
Nmic, g/gN		-	495 ± 21	437 ± 22	0.021	0.616	0.835
Nmic, g/kgDOM		-	17.7 ± 0.6	16.7 ± 0.6	0.051	0.180	0.368
Nmic, g/kgTDN		-	16.7 ± 0.6	17.9 ± 0.6	0.049	0.196	0.399

[1]NDF_{ap} = neutral detergent fiber corrected for ash and protein, Nmic = microbial N and DOM = digestible organic matter.

[2]FL= feeding level, DP = days of pregnancy and FL×DP = interaction between feeding level and days of pregnancy.

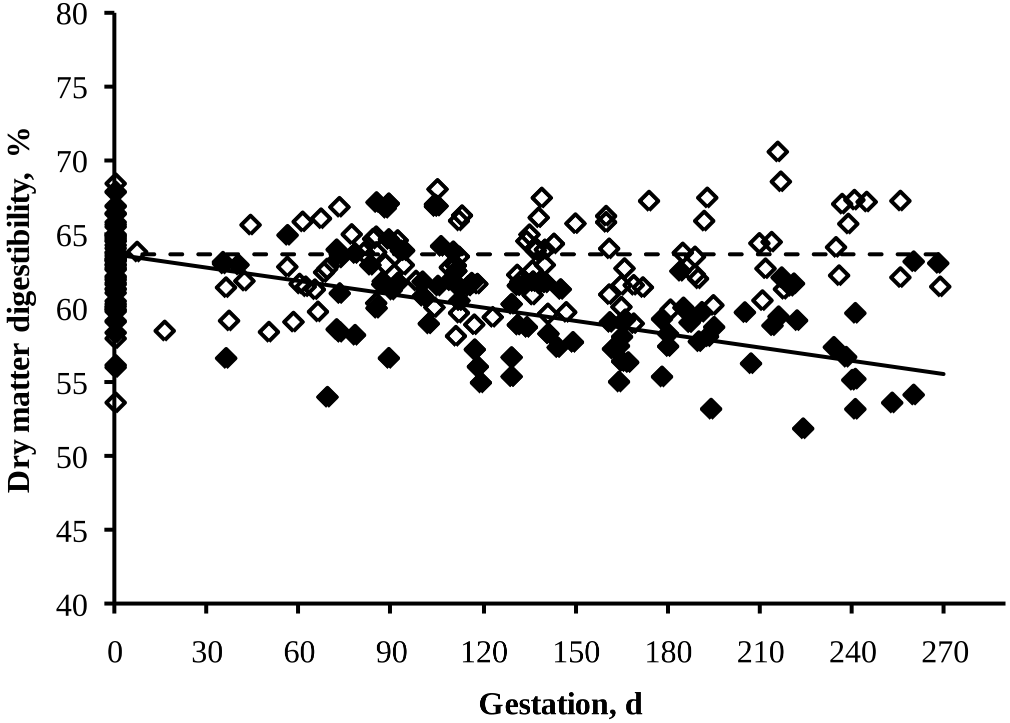
[3]The slope for days of pregnancy was not significant (P>0.10).

Figure 1



12
11
10
9
8
7
6
5
4
3
2
1
0
DMI, kg/d
0
30
60
90
120
150
180
210
240
270
Gestation, d

Figure 2



80
75
70
65
60
55
50
45
40
Dry matter digestibility, %
0
30
60
90
120
150
180
210
240
270
Gestation, d

CHAPTER 4

Energy and protein requirements for pregnant and non-pregnant Nellore cows

ABSTRACT: Fourty-nine adult Nellore cows (32 pregnant and 17 non-pregnant) with average initial body weight of 451 ± 10 kg were used in a comparative slaughter study aiming to describe equations and relationships for prediction of net, metabolizable and dietary energy and protein requirements for adult, pregnant and non-pregnant, *Bos indicus* cows. Feeding control was measured individually and cattle were fed either HIGH (*ad libitum*) or LOW (restricted feeding 1.2 times maintenance according to the NRC). The 32 pregnant cows were separated at random into 4 groups of 8 cows each (4 cows per each feeding level) and harvested at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. The non-pregnant cows were harvested at different times of the experiment (85 to 216 days of feeding control) in order to keep them in experiment for a similar amount of time as the pregnant cows. The digestible energy and N and losses of energy as methane and urine were directly measured to establish the relations between GE, DE and ME. Energy and N content were analyzed in empty body and pregnant compounds and a set of relationships and equations based in the factorial method from ARC was used to estimate the nutrient requirements of energy and N. The net energy and protein requirements for pregnancy (NE_p and NP_p) estimated in this study were about ¾ of those estimated by NRC. When estimated by a logistic model, the daily requirements for pregnancy showed an exponential increase up to approx. 250 days of gestation and then decreased. However, when an allometric model was used to estimate the daily requirements for pregnancy, the maximum daily requirements were at birth. There were no differences in the dynamics of energy and protein (P=0.388 and 0.137, respectively) in the cow's empty body weight pregnant free (EBW_{np}) suggesting that the pregnancy does not affect the requirements for accretion of body reserves in cows. The partial efficiencies for use of metabolizable energy for maintenance, weight gain and pregnancy (k_m, k_g and k_c) were respectively 70, 53 and 12%. The partial efficiencies for use of metabolizable protein for maintenance, weight gain and pregnancy (z_m, z_g and z_c) were respectively 22, 22 and 5%, indicating that in mature cows the major portion of metabolizable protein intake is not used for tissue accretion. The efficiency of transformation of DE in ME was 0.80.

KEYWORDS: beef cattle, *Bos indicus*, conceptus, metabolizable energy, metabolizable protein

RESUMO: Quarenta e nove vacas Nelore adultas (32 gestantes e 17 não gestantes) com peso médio inicial de 451 ± 10 kg foram usadas num experimento de abate comparativo objetivando descrever equações e relações para predizer as exigências líquidas e metabolizáveis de energia e proteína para vacas zebuínas adultas, gestantes e não gestantes. O consumo de alimento foi mensurado individualmente, sendo as vacas alimentadas com alto (ad libitum) ou baixo nível alimentar (1,2 vezes a mantença, segundo o NRC). As 32 vacas gestantes foram separadas em 4 grupos com 8 vacas cada (4 de cada nível alimentar) e abatidas aos 136 ± 1, 189 ± 1, 239 ± 1 e 269 ± 1 dias de gestação. As vacas não gestantes foram abatidas em diferentes tempos de experimento (85 a 216 dias de controle experimental) objetivando mantê-las em experimento por tempo semelhante às vacas gestantes. A energia e N digestível, bem como as perdas de energia na forma de metano e urina foram diretamente mensuradas para estabelecer as relações entre energia bruta, energia digestível e energia metabolizável. Os conteúdos de energia e N foram diretamente analisados no corpo vazio e no componente gestação das vacas. Um conjunto de equações e relações baseadas no método fatorial foi utilizado para estimar as exigências nutricionais de energia e proteína. Os requerimentos líquidos de energia e proteína para gestação (EL_p e PL_p) estimados nesse estudo foram em média ¾ dos do NRC. Quando estimados por um modelo logístico, os requerimentos para gestação aumentaram até aproximadamente 250 dias de gestação, e então reduziram. Entretanto, quando estimados por modelos alométricos simples, os requerimentos para gestação atingiram o máximo somente próximo ao parto. Não foram observadas diferenças na dinâmica de energia e proteína (P=0,388 e 0,137, respectivamente) no corpo vazio da vaca livre do componente gestação, sugerindo que a gestação não afeta as exigências nutricionais para acumulo de reservas corporais em vacas adultas. As eficiências parciais de uso da energia metabolizável para mantença, ganho de peso e gestação (k_m, k_g e k_c) foram, respectivamente, 70, 53 e 12%. As eficiências parciais de uso da proteína metabolizável para mantença, ganho de peso e gestação (z_m, z_g e z_c) foram respectivamente 22, 22 e 5%, indicando que em vacas adultas grande parte da proteína metabolizável não é recuperada na forma de tecidos. A eficiência de transformação da energia digestível em energia metabolizável foi 0,80.

Palavras-chave: abate comparativo, *Bos indicus*, energia metabolizável, gado de corte, proteína metabolizável

INTRODUCTION

Meeting the nutritional requirements for pregnant beef cows is important to ensure an adequate supply of nutrients for growth and development of the fetus. Likewise, it is also important to ensure to cow an appropriate body condition at parturition, lactation, and the beginning of a new pregnancy in about 90 days.

According to Ritchie (1995) 71% of the total energy spent in the beef production cycle is used for maintenance. About 70% of this energy is used for maintenance of the breeding herd. Therefore, 50% of the energy used in beef production is used for the maintenance of cows. Thus, variations in feed efficiency of these groups of animals have great impact on the production cycle.

Studies using comparative slaughter to estimate the nutrient requirements of pregnant cows are limited. The main feeding systems (ARC, 1980; AFRC, 1993; NRC, 2000; INRA, 2007; CSIRO, 2007) have their recommendations based on a few works of in indirect estimates and adjustments of values obtained from experiments with sheep. No studies about nutrient requirements of pregnancy in *Bos indicus* cattle are available.

The weight and chemical composition of fetus and gravid uterus are the main determinants of nutritional requirements for pregnancy. The breed or genotype of the bull and cow are the most influential factors on calf weight at birh (Andersen e Plum, 1965). Considerable differences are observed in the chemical composition of *B. indicus* (Fonseca, 2009) and *Bos taurus* calves (Cano, 1995) at birth.

The objective of this study was to describe equations and relationships for prediction of net, metabolizable and dietary energy and protein requirements for adult, pregnant and non-pregnant, Nellore cows.

MATERIALS AND METHODS

This study was conducted at the Federal University of Viçosa (Viçosa, MG, Brazil), following the standard procedures for humane animal care and handling according to the guidelines of Federal University of Viçosa (Brazil).

Animals

Forty-nine multiparous Nellore cows with average initial body weight of 451 ± 10 kg, age of 5.6 ± 0.5 years and body condition score of 4.4 ± 0.2 (1 to 9 scale) were obtained from Federal University of Viçosa herd and commercial sources. Animals were from an experiment reported by Gionbelli et al. (2013). From the initially 49 cows, 32 were separated at random and hand mated with Nellore bulls to form the pregnant group. Twelve cows were separated at random and assigned to the non-pregnant group and the 5 remaining cows were designated as the baseline group and harvested.

Diet and management

Cows were housed in pens (48 m², 6 cows per pen) with a concrete floor, 15 m² of covered area, and *ad libitum* access to fresh water. Feed intake was measured individually using an electronic head gate system (Kloppen Soluções Tecnológicas, Pirassununga, SP, Brazil). Bred cows were at 47 ± 3 d of gestation when the feeding trial started.

All cows were fed the same diet twice daily (0700 and 1500 h) and segregated into 2 groups according to level of feeding, either **HIGH** (*ad libitum*) or **LOW** (restricted feeding 1.2 times maintenance according to the NRC). Sixteen pregnant and 5 non-pregnant cows were fed HIGH level and 16 pregnant and 7 non-pregnant cows were LOW-fed. The restricted feeding was estimated so that 1.2 times maintenance sustained pregnancy and the HIGH-fed allowed some maternal tissue storage. The diet consisted of corn silage, ground corn, soybean meal, urea and mineral mixture (Table 1).

The 32 pregnant cows were separated at random into 4 groups of 8 cows each (4 cows per each feeding level) and harvested at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. The non-pregnant cows were harvested at different times of the experiment (85 to 216 days of feeding control) in order to keep them in experiment for a similar amount of time as the pregnant cows. The baseline cows were harvested on d 0 of the experiment after the end of the adaptation period and were used to estimate the initial chemical composition of the cows in the experiment.

Digestible energy and N

The amount of DE an digestible N of the diet was estimated as the coefficient of the total tract disappearance (intake minus excretion) and intake of GE and N. Daily fecal excretion was estimated using indigestible NDF (iNDF) as an internal marker (Lippke et al., 1986) of the digesta. Fecal samples were collected from each cow during 5 consecutive d (at 1800, 1500, 1200, 0900 and 0600 h for the first to fifth d, respectively) in 9 collection periods. Fecal samples (60 g of wet weight of each d of collection) were pooled for each animal in each period and stored at -20ºC for subsequent analyses.

Urinary energy and nitrogen

Two spot samples of urine were collected from each cow in the same periods of fecal collection at the second and the fifth d of fecal sampling, at 1600 and 0800 h, respectively. Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine excretion (mg/d) of $37.88 \times SBW^{0.9316}$ (Santos et al., unpublished data). The urinary concentrations of creatinine, allantoin and uric acid were obtained according George et al. (2006). Urine samples were stored at -20ºC for subsequent analyses of GE and N.

Methane production

A trial to estimate the methane emissions was performed using 5 (3 maintenance and 2 ad libitum) cows with 561 ± 67 (477 to 639) kg of shrunk body weight and 188 ± 58 (131 to 260) days of pregnancy. The trial was performed during the experiment and cows were fed with the same diet in the normal conditions of the experiment. Cows were transferred to individual pens (48 m^2, concrete floor, 15 m^2 of covered area), mobile feeders were introduced in the pen and the access to the automatic feeders was temporarily restricted.

The SF_6 tracer technique was used to measure methane emissions (Johnson et al., 1994). Seven days before the beginning of measurements a brass permeation tube (12.5 × 40 mm) that released utra-pure SF_6 as a rate of 3.47 mg/d was placed in the rumen of each animal using a polyvinylchloride probe placed in the esophagus of the cows. The trial was performed 10 days before slaughter so that the brass permeation tubes could be recovered at slaughter. Four day before the beginning of measurements each cow was fitted with a halter and extension tugging to draw air from the nostril area into an evacuated polyvinylchloride head canister that was attached to the animal's neck for gas sampling. All canisters were removed each day at the same time to collect gas samples. Technical information of the equipment, sampling, methane and SF_6 analyses and methane emission calculations were the same that those described by Beauchemin et al. (2012).

Body condition score

Body condition scores (**BCS**) were assessed on a scale ranging from 1 = severely emaciated to 9 = very obese (Nicholson and Butterworth, 1986; Richards et al., 1986) with 0.5 of partial scoring and were determined by observation and palpation. The trial for determining the BCS of each cow was performed before harvest by two observers in a double-blind scheme. Each observer did not know the result of the evaluation of the other, and there was calculated the average score or performed a new evaluation if the scores of the two evaluators were far more than 1.5 points.

Animal harvest and sampling

Pre-harvest animal care and handling procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). Before harvest, feed was withheld from animals for 16 h, but they had *ad libitum* access to water the entire time. Harvest was performed by electrical stunning followed by exsanguination.

At harvest, udders were removed along with the portion of hide that covered the udder. Udders were weighed, ground, and samples were taken for chemical analyses. The Uterus were severed at the cervix and collected for analyses. The gravid uterus was weighed and dissected into the fetus, the uterus and ovaries, and the placenta and fetal fluids (allantoic and amniotic fluids). All of the gravid uterus components were individually weighed and the weights of the fetal fluids were calculated by difference. Samples of fetal fluids were collected for chemical analyses. All of other components of the gravid uterus were ground in a bowl cutter (Refere Inox, Chapecó, SC, Brazil) and sampled for gross energy and N analyses.

The cow's whole body was segregated into head, legs, hide, organs and viscera, blood and carcass (lean, fat and bone). The grouping of all components of whole body after the wash of the digestive tract constituted the pregnant empty body weight (**EBW_p**, including udder and gravid uterus of pregnant cows) or the empty body weight (**EBW**, for non-pregnant cows). The EBW in a non-pregnant condition (**EBW_{np}**) of pregnant cows was calculated according Gionbelli et al. (2013). All of non-carcass compounds (head, members, blood, hide, organs and viscera) were ground and one sample was taken for chemical composition analyses. The carcasses were weighed and chilled in a cold chamber (4 ºC) for 24 h. After chilling, the carcasses were weighed and separated into bone and soft tissue (lean and fat). All carcass tissues were ground, grouped and sampled for chemical composition analyses. The soft tissues components were ground using a bowl cutter and the rigid tissues were ground initially in a industrial grinder, and after in a bowl cutter. The homogenization and the grouping to make the carcass and non-carcass composite samples also were performed using a bowl cutter. All samples were stored frozen (-80 ºC) until chemical analyses could be performed.

Chemical analyses

All samples were lyophilized. The feed, orts and feces samples were ground using a Willey mill (TE-650, Tecnal, Piracicaba, SP, Brazil) to pass a 1 mm screen. The samples of animal tissues were frozen-powdered using liquid nitrogen and a blender. The DM was collected by oven drying at 100 ºC for 18 hours (Method 934.01; AOAC, 2000). The CP content was calculated based on the N content multiplied by the 6.25 factor. The N content was calculated by Kjeldahl procedure (Method 920.87; AOAC, 2000). Gross energy was measured using an adiabatic bomb calorimeter (C5001, IKA-Werke Co, Staufen, Germany). For iNDF analysis, the samples of feed, orts and feces were incubated in situ for 10 d (Casali et al., 2008) in the rumen of 3 Holstein×Nellore cows that had ad libitum access to the same diet used in the experiment. Residual samples were analyzed for the NDF. The NDF content was obtained according to method described by Van Soest et al. (1991), with addition of sodium sulfite (Undersander et al., 1993) for analyses of soybean meal, because of the gelatinization of the protein content. Ankom 200 system (Ankom, Macedon, NY) was used for the NDF evaluations, with heat-stable α-amylase addition and modification of the bag utilized (5.0 × 5.0 cm, 100 μm porosity, Casali et al, 2009), which was manufactured using nonwoven fabric tissue (100 g/m^2). In all the samples NDF content was corrected for ash and protein.

Metabolizable energy and protein intake

Metabolizable energy intake (**MEI**) was calculated by the DE intake minus urinary and gaseous energy losses. Metabolizable protein intake (**MPI**) was estimated as the intake of digestible microbial protein plus the ruminal undegradable protein (**RUP**). We assumed that the microbial crude protein contains 80% of amino acids and a small intestine digestibility of 80% (NRC, 2000). A fixed value of 80% was also used for the digestibility of RUP in the small intestine (Marcondes et al. 2010). The values of digestible organic matter and microbial protein production for cows of this study were obtained from Gionbelli et al. (2013c).

Nutrient requirements calculations

To estimate the recovered amounts of energy and N (**RE** and **RN**) related to pregnancy and cows body tissues we adopted the concept of pregnant compound (**PREG**) presented by Gionbelli et al. (2013). The PREG represents the amount of some component that is genuinely related to pregnancy, that includes the gravid uterus minus the non-pregnant uterus plus the accretion in udder related to pregnancy. Thus, the recovered amounts of energy and N in the cow's tissues free from pregnancy (CT) are the amounts in carcass, offal, hide, blood, head, feet, udder, plus the non-pregnant uterus minus the accretion in udder related to pregnancy. The recovered amounts of energy and N in the whole cow's body (WB) are then the sum of CT and PREG. The concept of PREG does not address, however, the possible variations that occur in the other cow's body components as bone, muscle, adipose tissue and offal. These variations, though actually happen due to the homeorhetic effect (Hammond, 1947) are extremely difficult to modelling.

The nutrient requirements of energy and N for pregnant and non-pregnant Nellore cows were estimated using factorial procedures (ARC, 1965). A complete description of the relationships and abbreviations used is described in Table 2. Details and discussion about the equations used are made along with the presentation of the results. The energy terms were based in NRC (1981) recommendations.

Statistical Procedures

The procedures REG and GLM of SAS (SAS version 9.2, SAS Inst. Inc., Cary, NC) were used to estimate the regression parameters of linear functions. Parameters for nonlinear functions were estimated using the Gauss-Newton method in NLIN or NLMIXED procedures of SAS. When appropriate, the degrees of freedom were adjusted using the Kenward-Roger method. The value of 0.05 was adopted as critical level of probability for occurrence of Type I error. Probability levels between 0.05 and 0.10 were discussed as tendency.

The basic mathematical model used to estimate the dynamics of recovered energy or N in PREG in function of time of pregnancy was of the logistic form, similar to Koong (1975):

$$RC_p = RC_0 \times e^{(\beta_1 + \beta_2 \times DIP + \beta_3 \times BCS) \times DIP} \quad [1]$$

where RC_p = the recovered component (energy or N) in the pregnant compound, RC_0 = the recovered component (energy or N) in the pregnant compound on day 0 of pregnancy, DIP = days in pregnancy and BCS = body condition score.

Alternatively, the dynamics of recovered energy or N in PREG in function of time of pregnancy were adjusted to an allometric non-linear model as follow:

$$RC_p = \beta_1 \times DIP^{\beta_2} \quad [2]$$

where RC_p = the recovered component (energy or N) in the pregnant compound and DIP = days in pregnancy.

The mathematic model used to estimate the dynamics of udder energy and N in function of gestational age was based in the work of Fadel (2004), as a segmented non-linear model with a period of staticity followed by an exponential model, as follow:

a. If DIP < B_0 then $UD = SBW_{np} \times B_1 \times BCS^{B_2}$

b. If DIP > B_0 then $UD = SBW_{np} \times B_1 \times BCS^{B_2} \times e^{[B_3 \times (DIP - B_0)]}$

[3]

where: DIP = days in pregnancy, UD = udder energy or N content (kg), SBW_{np} = shrunk body weight of a cow in non-pregnant condition (kg) and BCS = body condition score. The parameter B_0 represents the moment in which the energy or N content of the udder starts to increase in function of days of pregnancy. The model *a* is used to estimate the energy or N content of the udder of non-pregnant cows and the use of *a* or *b* model is used to pregnant cows depending on the values of B_0 and DIP.

The equations to predict methane emissions and urinary energy or N were made using a mixed model (MIXED procedure of SAS) that included the fixed effect of energy, N or dry matter intake, the random effect of animal, and the repeated effect of day (methane) or period (urinary energy and N). Because the interval between collections periods varied the urinary energy and N are considered to be measured irregularly. Under these circumstances a continuous-time model was used to describe the covariances among the errors as proposed by Moser (2004). The spatial data covariance structures provided in PROC MIXED of SAS was used. The spatial power was used as covariance function. Parameters were considered significant at P<0.10.

Model evaluations

When two or more models were generated to predict the same variable, the choice of the best model was based on Akaike information criterion (AIC). The AIC is a procedure to identify the best fit to the model, which consider the number of parameters and make the evaluation free from ambiguities associated with conventional hypothesis testing (Akaike, 1974). The AIC was estimated as follows (Kaps & Lamberson, 2004):

$$AIC = n \times log(SS_{RES}/n) + 2 \times p \quad [4]$$

were SS_{RES} = residual mean square, n = the number of observations and p = the number of parameters of the model.

To verify the effect of pregnancy on dynamics of non-pregnant tissues functions from pregnant and non-pregnant dataset were predicted using the same model. For each function, an *F*-ratio was calculated to test whether estimation of parameters specific to each physiological group significantly improved fit of the data relative to estimation of parameters from a pooled data set, ignoring the pregnancy effect. The test statistic was as follows:

$$F = \frac{(RSS_{P} - RSS_{PR} - RSS_{NP})/(Rdf_{P} - Rdf_{PR} - Rdf_{NP})}{(RSS_{PR} + RSS_{NP})/(Rdf_{PR} + Rdf_{NP})}$$

where RSS represents residual sums of squares and Rdf denotes residual degrees of freedom; the subscripts P, PR and NP indicated pooled model, pregnant and non-pregnant cows, respectively. A *P*-value for the F distribution was used to evaluate if single model was inappropriate and that the dynamics of traits differs between physiological status.

For predicted functions, the deviations of the predicted from observed values were regressed as function of the predicted values using the REG procedure of SAS. Model was considered feasible when β_0 and β_1 were not different from zero (P>0.05). The significance of the means of the deviations between observed and predicted values was evaluated using the MEANS procedure of SAS and the model was considered feasible when the mean was not different from zero (P>0.05).

To verify the effectiveness of the generated models, the observed values were regressed in function of predicted values and the hypothesis that $\beta_0 = 0$ and $\beta_1 = 1$ was evaluated (Mayer et al., 1994). When appropriate, the mean square error of prediction (**MSEP**, Bibby and Toutenburg, 1977) and the partition of sources of variation of MSEP (Theil, 1961) were also evaluated. These statistics were calculated using the Model Evaluation System (MES, v.3.0.1, http://nutritionmodels.tamu.edu/mes.htm) as proposed by Tedeschi (2006).

RESULTS AND DISCUSSION

Recovered energy in pregnant compound and in empty body

The recovered energy or N related to the pregnancy (**RE_p** and **RN_p**) were estimated based on the theoretical assumption that the total RE_p and RN_p are the amounts of energy and N in the gravid uterus ($RE_{graviduterus}$ and $RN_{graviduterus}$) less the amounts in the non-pregnant uterus (that would be present even cow was non-pregnant) plus the accretion observed in udder due to pregnancy (i.e. the difference in amounts of energy and N in udder between a pregnant and non-pregnant cow with the same SBW_{np}).

Data from the 17 non-pregnant cows were used to predict the recovered energy and N in the non-pregnant uterus plus ovaries (**$RE_{nputerus}$** and **$RN_{nputerus}$**). The procedure of SAS did not converge when BCS was included in the model. The non-linear relationship was better than linear relationship (AIC = 61.1 vs 62.9 for $RE_{nputerus}$ and 14.9 vs 16.3 for $RN_{nputerus}$) to predict $RE_{nputerus}$ and $RN_{nputerus}$ from the SBW_{np}.

$$RE_{nputerus} = 0.4701 \pm 0.6 \times SBW_{np}^{1.1387 \pm 0.2056} \quad [5]$$

$$RN_{nputerus} = 0.000309 \pm 0.0004 \times SBW_{np}^{1.7378 \pm 0.2444} \quad [6]$$

where $RE_{nputerus}$ = recovered energy in non-pregnant uterus (kcal), $RN_{nputerus}$ = recovered N in non-pregnant uterus (g) and SBW_{np} = shrunk body weight in a non-pregnant condition (kg).

The use of days in pregnancy in the model to describe the recovered energy in the udder (**RE_{udder}**) showed no improvement. Although udders of pregnant cows increase in fresh weight in late gestation (P<0.10) there was no significant increase (P=0.35) in energy content of the udder (Mcal) with the increase of gestation in cows of this study (Gionbelli et al., 2013a). Thus we conclude that the dynamics that occur in udder during pregnancy do not affect the energy requirements for pregnancy. Nevertheless a model was generated to predict the RE_{udder}. The use of SBW_{np} showed better AIC (95.2) than the use of SBW (95.8) in the model. Inclusion of BCS beyond the SBW_{np} not improved the model (AIC = 96.4) and was not used.

$$RE_{udder} = 9.6 \pm 1.61 \times SBW_{np} 1.2939 \pm 0.2675 \qquad [7]$$

where RE_{udder} = recovered energy in udder (kcal) and SBW_{np} = shrunk body weight in a non-pregnant condition (kg).

The recovered N in udder (**RN_{udder}**) presented a relationship with the SBW and an exponential increase due to gestation after 238 days of pregnancy. The point where RN_{udder} start to increase due to pregnancy was the same found by Gionbelli et al. (2013b) for the udder fresh weight. The use of SBW_{np} instead SBW improve the model (AIC = -158.7 vs -163.5) and the linear relationship with SBW_{np} presented better AIC (-163.5) than non-linear relationship (-161.5) because the use of 3 parameters instead 4. Based on the model to predict RN_{udder} the RN_{udder} that occur due to the pregnancy (**$RN_{udderDP}$**) was calculated as follows:

If DIP≤238 then $RN_{udder} = 0.000164 \pm 000006 \times SBW_{np}$
If DIP>238 then $RN_{udder} = 0.000164 \pm 000006 \times SBW_{np} \times exp^{(0.0163 \pm 0.0035 \times DIP)}$ [8]

then,
If DIP>238 then $RN_{udderDP} = 0.000164 \times SBW_{np} \times exp^{(0.0163 \times DIP)} - 0.000164 \times SBW_{np}$ [9]

where RN_{udder} = recovered N in udder (g), SBW_{np} = shrunk body weight in a non-pregnant condition (kg), DIP = days in pregnancy and $RN_{udderDP}$ = recovered N in udder due to the pregnancy (kg).

Based on the equations [5] and [6] the amounts of $RE_{nputerus}$ and $RN_{nputerus}$ of pregnant cows were subtracted from the gravid uterus and added to recovered energy and N in empty body (**RE_{eb}** and **RN_{eb}**), and based on Eq. [9] the amounts of $RN_{udderDP}$

was subtracted from RN_{eb} of pregnant cows that were harvested after 238 days of gestation and added into the $RN_{graviduterus}$ to form the RE_p and RN_p. Summarily the way to calculate RE_p and RN_p can be described as follows:

$RE_p = RE_{graviduterus} - RE_{nputerus}$ and

If DIP $\leq$ 238 than $RN_p = RN_{graviduterus} - RN_{nputerus}$ or,

If DIP > 238 than $RN_p = RN_{graviduterus} - RN_{nputerus} + RN_{udderDP}$

At the same time, the recovered energy and N in the whole body minus the pregnant compound are defined as recovered energy and N in cow's tissues (**RE_{ct}** and **RN_{ct}**) and were calculated for pregnant cows as follows:

$RE_{ct} = RE_{eb} + RN_{nputerus}$ and

If DIP $\leq$ 238 than $RN_{ct} = RN_{eb} + RN_{nputerus}$ or,

If DIP > 238 than $RN_{ct} = RN_{eb} + RN_{nputerus} - RN_{udderDP}$

Because was an effect of feeding level on the weight of gravid uterus at 269 d of gestation in the cows of this study (Gionbelli et al., 2013a) the data from LOW-fed cows were removed from the dataset to estimate the net requirements of pregnancy. The non-linear logistic model (Eq. [1]) was which best fitted to describe RE_p. Although the inclusion of BCS in the model reduced the error sum of squares in 11%, the AIC increased from 36.6 to 36.8 because the increase of number of parameters. This means that BCS has some influence on RE_p. Gionbelli et al. (2013b) observed significant effect of BCS on gravid uterus fresh weight. The both models, with (Eq. [10]) or without BCS (Eq. [11]) were then adjusted. In both cases the regression of deviations of the predicted from observed values as function of the predicted values was not significant (P=0.578 and 0.539 for β_0 and 0.659 and 0.627 for β_1 for Eq. [10] and Eq. [11], respectively). The mean of the deviations between observed and predicted values was also not different from zero (P=0.730 and 0.703 for Eq. [10] and Eq. [11], respectively).

$$RE_p = 0.2777{\pm}0.7206 \times exp^{((0.07761\pm0.0219 - 0.0001311\pm0.000046 \times DIP + 0.0001545\pm0.000127 \times BCS) \times DIP)} \quad [10]$$

$$RE_p = 0.4444{\pm}1.1460 \times exp^{((0.07365\pm0.0217 - 0.0001186\pm0.000046 \times DIP) \times DIP)} \quad [11]$$

where RE_p = recovered energy in pregnant compound (kcal), DIP = days in pregnancy and BCS = body condition score.

The RE_p and RN_p were highly correlated (Figure 1) and because of this the same characteristics observed modeling RE_p were observed modeling RN_p. Thus, models including (Eq. [12]) or not including BCS (Eq. [13]) were fitted.

$$RN_p = 0.01999{\pm}0.0474 \times exp^{((0.06841\pm0.0201 - 0.0001102\pm0.000043 \times DIP + 0.0001018\pm0.000122 \times BCS) \times DIP)} \quad [12]$$

$$RN_p = 0.02713{\pm}0.0629 \times exp^{((0.06583\pm0.0196 - 0.0001020\pm0.000041 \times DIP) \times DIP)} \quad [13]$$

where RN_p = recovered N in pregnant compound (g), DIP = days in pregnancy and BCS = body condition score.

Alternatively, allometric non-linear models (Eq.[2]) were fitted to the data of RN_e and RN_p (Eq. [14] and [15]). Inclusion of BCS in the models showed lack of fit and was not used. Residuals analysis of Eq. [14] and [15] showed adequate fit. The residuals means of both functions (-0.144 for Eq. [14] and -2.94 for Eq. [15]) were not different from zero (P=0.807 and P=0.828, respectively). The intercept and slope of residuals in function of predicted values of Eq. [14] and [15] were not different from 0 (P=0.682 and 716 for the intercepts and P=0.740 and 769 for the slopes, for Eq. [14] and [15], respectively).

$$RN_e = 0.000000013666 \pm 0.00000002 \times DIP^{3.8585 \pm 0.3310} \quad [14]$$

$$RN_p = 0.0000003421 \pm 0.0000005 \times DIP^{3.8550 \pm 0.3093} \quad [15]$$

The average predicted calf birth weight in this study was 28 kg. The Eq. [11], [13], [14] and [15] can be scaled for different expected calf birth weight. The Eq. [10]

and Eq. [12] were not scaled for calf birth weight because the BCS included in the model is suggested to be related to calf birth weight.

$$RE_p = 0.01587 \times calf\ birth\ weight \times exp^{((0.07365 - 0.0001186 \times DIP) \times DIP)} \quad [16]$$

$$RN_e = 0.0000000004881 \times DIP^{3.8585} \quad [17]$$

$$RN_p = 0.000969 \times calf\ birth\ weight \times exp^{((0.06583 - 0.0001020 \times DIP) \times DIP)} \quad [18]$$

$$RN_p = 0.00000001222 \times DIP^{3.8550} \quad [19]$$

where RE_p = recovered energy in pregnant compound (kcal), RN_p = recovered N in pregnant compound (g) and *DIP* = days in pregnancy.

The models to describe the RE_{ct} and RN_{ct} as function of EBWnp are described in Eq. [20] and Eq. [21]. The F-test was performed between functions estimated based on data from pregnant and non-pregnant cows and showed no effect of pregnancy on the RE_{ct} (P=0.388) and RN_{ct} (P=0.137). Thus, we concluded that the pregnancy does not affect the dynamics of energy accretion in maternal tissues.

$$RE_{ct} = 0.0731 \pm 0.0540 \times EBW_{np}^{1.6084 \pm 0.1201} \quad [20]$$

$$RN_{ct} = 58.05 \pm 12.26 \times EBW_{np}^{0.8645 \pm 0.0344} \quad [21]$$

where RE_{ct} = recovered energy in cow's body tissues (Mcal), RN_{ct} = recovered N in cow's body tissues (g) and EBW_{np} = empty body weight in a non-pregnant condition.

In both RE_{ct} and RN_{ct} the regression of deviations of the predicted from observed values as function of the predicted values was not significant (P=0.838 and 0.939 for β_0 and 0.876 and 0.918 for β_1 for Eq. [20] and Eq. [21], respectively). The mean of the deviations between observed and predicted values was also not different from zero (P=0.890 and 0.891 for Eq. [20] and Eq. [21], respectively).

Net requirements for pregnancy

The net requirements of energy and N for pregnancy (**NE_p** and **NN_p**) were obtained from the first derivative of Eq. [16] to [19], as follows:

$$NE_p = calf\ birth\ weight \times (0.001169 - 0.000003764 \times DIP) \times exp^{((0.07365 - 0.0001186 \times DIP) \times DIP)} \quad [22]$$

$$NE_p = calf\ birth\ weight \times 0.000000001883 \times DIP^{3.8585} \quad [23]$$

$$NN_p = calf\ birth\ weight \times (0.00006378 - 0.0000001977 \times DIP) \times exp^{((0.06583 - 0.0001020 \times DIP) \times DIP)} \quad [24]$$

$$NE_p = calf\ birth\ weight \times 0.00000004710 \times DIP^{3.8550} \quad [25]$$

were NE_p = net energy requirements for pregnancy (kcal/d), NN_p = net N requirements for pregnancy (g/d) and *DIP* = days in pregnancy.

Considering the nutrient requirements from 100 to 280 d of gestation, the total the NE_p estimated for *B. indicus* cattle are about ¾ (74% using logistic model of Eq. [22] and 75% using allometric model of Eq. [23]) than those estimated by NRC (2000). The proportions, however, varied throughout the gestation (Figure 2 and Figure 3). The NE_p and NN_p estimated by logistic model increased throughout the gestation till 252 and 246 days of gestation, respectively, and then start to decrease, differently from the estimated by the allometric functions (Eq. [23] and [25]) and from those observed in study of Ferrell et al. (1976a) when the maximum rate of deposition of energy and N were after birth.

Net requirements for weight gain

The net requirements of energy and protein for weight gain (**NE_g** and **NP_g**) were firstly obtained from the first derivative of Eq. [20] and Eq. [21], as follows:

$$NE_g = rEBG \times 0.1176 \times EBW_{np}^{0.6084} \quad [26]$$

$$NP_g = rEBG \times 313.6 \times EBW_{np}^{-0.1355}$$ [27]

where NE_g = net requirement of energy for gain (Mcal/d), NP_g = net requirement of protein for gain (g/d), EBW_{np} = empty body weight in a non-pregnant condition and rEBG = real empty body gain (kg/d).

Net energy for weight gain was also estimated as a function of $EBW_{np}^{0.75}$ and rEBG similar NRC (2000) and BR-CORTE (Valadares Filho et al., 2010), as follows:

$$NE_g = 0.1063 \pm 0.0464 \times EBW_{np}^{0.75} \times rEBG^{0.8302 \pm 0.1023}$$ [28]

where NE_g = net requirement of energy for gain (Mcal/d), EBW_{np} = empty body weight in a non-pregnant condition and rEBG = real empty body gain (kg/d).

Net protein for weight gain was also estimated as function of $EBW_{np}^{0.75}$ and rEBG or as function of NE_g and rEBG (NRC, 2000), as follows:

$$NP_g = 1.3431 \pm 0.0975 \times EBW_{np}^{0.75} \times rEBG^{1.0831 \pm 0.1567}$$ [29]

$$NP_g = 208 \pm 17 \times rEBG - 2.14 \pm 0.48 \times NE_g$$ [30]

where NP_g = net requirement of protein for gain (g/d), EBW_{np} = empty body weight in a non-pregnant condition, NE_g = net requirement of energy for gain (Mcal/d) and rEBG = real empty body gain (kg/d).

Energy requirements for maintenance

The net energy for maintenance (NE_m) was estimated as the intercept of HP in function of MEI using only data from non-pregnant cows, as follows:

$$HP = 72.37 \pm 9.64 \times exp^{(MEI \times 0.00341 \pm 0.00094)}$$ [31]

where HP = heat production (kcal/$EBW^{0.75}$/d) and MEI = metabolizable energy intake (kcal/$EBW^{0.75}$/d). Thus, the NE_m can be calculated as follows:

$$NE_m = 72.37 \times EBW^{0.75}$$ [32]

where NE_m = net energy for maintenance (kcal/d) and EBW = empty body weight.

The requirements of metabolizable energy for maintenance (ME_m) were calculated by iteration of Eq. [31] to the point that MEI = HP. The value obtained for ME_m was 103.5 kcal/EBW$^{0.75}$/d. Thus, the ME_m can be calculated as follows:

$$ME_m = 103.5 \times EBW^{0.75} \quad [33]$$

where ME_m = metabolizable energy for maintenance (kcal/d) and *EBW* = empty body weight.

The partial efficiency of use of metabolizable energy for maintenance (k_m) was then obtained as the ratio of NE_m / ME_m that was 72.37 / 103.5 = 0.7. It's difficult to modeling the metabolizable requirements for maintenance in pregnant cows. As the same way of Ferrell et al. (1976) we assumed that the k_m does not vary between pregnant and non-pregnant cows. Robinson et al. (1980) suggested that k_m in pregnant animals is similar to other categories.

Protein requirements for maintenance and efficiency of use of metabolizable protein for weight gain

The net protein for maintenance (NP_m) was considered the intercept of the RP_{ct} regressed as function of metabolizable protein intake (MPI). The F-test showed no differences (P=0.324) between pregnant and non-pregnant cows for the parameters of the model. Thus, we concluded that the pregnancy does not affect the dynamics of protein usage for basal metabolism in Nellore cows.

$$RP_{ct} = -66.9 \pm 31.7 + 0.2155 \pm 0.0501 \times MPI \quad [34]$$

where RP_{ct} = net recovered protein in cow's body tissues (g/d) and *MPI* = metabolizable protein intake (g/d). To estimate the NP_m the intercept (66.9) was divided by de average EBW of the cows used to fit the function (66.9 / 422.4 = 0.158). Thus NP_m (g/d) = 0.158 × EBW.

The predicted function (Eq. [34]) showed a lower slope (0.2155), which means that adult cows have lower efficiency to transform metabolizable protein into net protein (z_g). This probably occurs due to mature cows had lower accretion of protein in gain than growing cattle, as visualized by the efficiency to transform metabolizable protein into net protein presented in BR-CORTE (Marcondes et al., 2010).

The metabolizable protein for maintenance (MP_m) was obtained by iteration of Eq. [34] to the point that RP_{ct} was equal to zero. The obtained value was 310.6 (g/d). This value was divided by the average EBW (422.4) of cows used in the model to obtain the value of MP_m, which was: MP_m (g/d) = 0.735 × EBW. The use of this way to calculate the values of NP_m and MP_m assumes that the efficiency of use of metabolizable protein for maintenance (z_m) is equal to z_g, which was 0.2155.

Metabolizable energy for weight gain

The partial efficiency of use of metabolizable energy for weight gain (k_g) was estimated as the slope of net RE_{ct} (kcal/$EBW^{0.75}$/d) as function of MEI (kcal/$EBW^{0.75}$/d) using only data from non-pregnant cows, as follows:

$$RE_{ct} = -63.5 \pm 13.9 + 0.5292 \pm 0.0813 \times MEI \quad [35]$$

where RE_{ct} = net recovered energy in cows tissues (kcal/$EBW^{0.75}$/d) and MEI = metabolizable energy intake (kcal/$EBW^{0.75}$/d). The slope of 0.5292 was used as k_g to predict the requirements of metabolizable energy for weight gain (ME_g), as the relation of $ME_g = NE_g / k_g$.

Metabolizable protein for weight gain

The value of metabolizable requirements of protein for weight gain is obtained by the division of the NP_g (Eq. [29] and [30]) by the z_g (slope of Eq. [34]).

Metabolizable energy for pregnancy

The partial efficiency of use of metabolizable energy for pregnancy (k_c) was estimated assuming that k_c = NE_p / (MEI – ME_m – ME_g) and using in dataset only data of pregnant cows. The ME_m was estimated for each cow using Eq. [33]. The individuals estimative of k_c were very variables. Thus, to estimate the average value of k_c, the daily amounts of NE_p, MEI, ME_m and ME_g (Mcal/d) were added, generating the following computation:

$k_c = NE_p / (MEI – ME_m – ME_g)$

$k_c = 2.67 / (490.39 – 316.82 – 151.42)$

$k_c = 2.67 / 22.15$

$k_c = 0.1205$

The value of 12% for efficiency of use of metabolizable energy for pregnancy is very close to efficiency of 14% found by Ferrell et al. (1976) and adopted by NRC (2000). It's suggested that the lower value of k_c is due to the higher level of metabolism of the placenta. According to Graham (1964) and ARC (1980) the value of k_c is usually higher in sheep (about 20%) than cattle, with has an average of 13%. The second model proposed to estimate k_c (k_{c2} in Table 2) showed lack of fit (P=0.656) and was not used.

Metabolizable protein for pregnancy

The partial efficiency of use of metabolizable protein for pregnancy (z_c) was estimated by the same way which was used to estimate k_c. Thus, assuming that z_c = NP_p / (MPI – MP_m – MP_g) and using in dataset only data of pregnant cows. The MP_m was estimated for each cow using MP_m (g/d) = 0.735 × EBW, as described previously. Thus, to estimate the average value of z_c, the daily amounts of NP_p, MPI, MP_m and MP_g (g/d) were added, generating the following computation:

$z_c = NP_p / (MPI – MP_m – MP_g)$

$z_c = 65.83 / (17892.29 – 14817.28 – 1775.96)$

$z_c = 65.83 / 1299.05$

$z_c = 0.0507$

The efficiency of use of metabolizable protein for pregnancy was wery low (z_c = 5%). The low value may be result of an extensive metabolism of protein in the gravid uterus. It's suggested that in late gestation about 55% of the energy used by gravid uterus is provided as amino acids (Bell et al., 2005) beyond those which are used to form protein. We suggest that the value of 5% of z_c is correct because there is some physiologic foundation. The second model proposed to estimate z_c (z_{c2} in Table 2) showed lack of fit (P=0.600) and was not used.

Methane production

The methane production of cows ranged from 106.6 L/d (77.3 g/d or 944 kcal/d) to 200.4 L/d (145.3 g/d or 1774 kcal/d) in this study. This represents from 5.27 to 9.29 percent of the gross energy ingested and from 8.92 to 14.9 percent of digestible energy ingested. The methane production was 124 (± 37) L/d for maintenance cows and 165 (± 48) L/d for cows fed ad libitum. The use of SF_6 technique appeared to be a high variable technique to estimate methane production when compared to the use of closed-circuit respiration equipment (Blaxter and Clapperton, 1965) and open-circuit respiration boxes (Freetly and Nienaber, 1998). High variability using SF_6 was also reported in other studies (Beauchemin et al., 2012; Pinares-Patiño et al., 2011).

Equations [36] to [46] were developed to predict methane production of cows (Table 3). Due to number of animals used in this study there was not possible to estimate possible effects of pregnancy on methane production. As the intake of energy increases it can be observed decrease in energy lost as methane in proportion of ingested energy in agreement of a meta-analysis from 207 cattle diets (Ramin and Huhtanen, 2013). The average methane production for LOW and HIGH-fed cows in this experiment (4.60 and 7.62 kg/d of DMI, respectively, Gionbelli et al., 2013c) was 133 and 172 L/d. The predicted methane production using Ramin and Huhtanen (2013) equation was 178 and 229 L/d.

Although the equations presented in Table 3 are very useful for practical use, no variables were significant to model the energy lost as methane as percentage of the DE intake. Thus, we estimated the mean of this proportion, which was 11.31±0.81 % of DE. This estimative was slightly greater than the average of values reported in previous studies (Blaxter, 1969; Blaxter and Clapperton, 1965).

Urinary energy losses

Even urinary energy losses (UE) have been evaluated in each cow at different times of pregnancy and at different feeding levels; we did not found significant relationship with variables to modeling the UE (% of DE). The proportion of DE lost as energy in urine was 8.45±0.74%. This value is within the range suggested by Blaxter and Clapperton (1966).

Efficiency of transformation of DE in ME

Using the percentages of DE that are lost as methane and urine (11.31 and 8.45%) we estimated the efficiency of transformation of DE in ME, which was:

EM / DE = (100 – UE – EE) / 100

EM / DE = (100 – 8.45 – 11.31) / 100

EM / DE = (80.24) / 100

EM / DE = 0.8024

This means that about 20% of DE is lost as methane and urine. The value of 0.8024 is similar to the value of 0.82 commonly adopted by almost all feeding systems. Hence, we suggest the adoption of 0.8024 as efficiency of transformation of DE in ME in *B. indicus* cattle.

Total metabolizable requirements

Based in the assumptions showed in Table 2 and in equations previous described the total metabolizable energy requirements (TME, Mcal/d) for pregnant and non-pregnant cows, at different BW and accumulating or not body reserves were estimated (Table 4). The partitioning of metabolizable energy requirements of a 450 kg BW cow is showed in Figure 4. The same path can be traced to calculate the total metabolizable protein requirements (TMP).

LITERATURE CITED

AFRC. 1993. Energy and protein requirements of ruminants. Agricultural and Food Research Council. CAB International, Wallingford, UK.

Akaike, H. 1974. A new look at the statistical model indentification. IEEE transactions on Automatic Control AC-19: 716-723.

Andersen, H., and M. Plum. 1965. Gestation length and birth weight in cattle and buffaloes: A review. Journal of Dairy Science 48: 1224-1235.

ARC. 1965. The nutrient requirements of farm livestock. Agricultural Research Council, London, UK.

ARC. 1980. The nutrient requirements of ruminant livestock. Agricultural Research Council. The Gresham Press, London.

Beauchemin, K. A., T. Coates, B. Farr, and S. M. McGinn. 2012. Technical note: Can the sulfur hexafluoride tracer gas technique be used to accurately measure enteric methane production from ruminally cannulated cattle? Journal of Animal Science 90: 2727-2732.

Bibby, J., and H. Toutenburg. 1977. Prediction and improved estimation in linear models. John Wiley & Sons, Berlin, Germany.

Blaxter, K. L. 1969. The efficiency of energy transformation in ruminants. In: Proceedings of Energy Metabolism of Farm Animals, 4, Warsaw. p 21-28.

Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. British Journal of Nutrition 19: 511-522.

Blaxter, K. L., J. L. Clapperton, and A. K. Martin. 1966. The heat of combustion of the urine of sheep and cattle in relation to its chemical composition and to diet. British Journal of Nutrition 20: 449-459.

Cano, J. G. 1995. The INRA systems of nutritional requirements of cattle. . In: SIMPÓSIO INTERNACIONAL SOBRE EXIGÊNCIAS NUTRICIONAIS DE RUMINANTES, Viçosa, MG.

CSIRO. 2007. Nutrient requirements of domesticated ruminants. Commonwealth Scientific and Industrial Research Organization, Collingwood, VIC.

Fonseca, M. A. 2009. Exigências nutricionais de vacas nelore em lactação e de bezerros, do nascimento à desmama, Universidade Federal de Viçosa, Viçosa, MG.

Freetly, H. C., and J. A. Nienaber. 1998. Efficiency of energy and nitrogen loss and gain in mature cows. Journal of Animal Science 76: 896-905.

Gionbelli, M. P., M. S. Duarte, S. C. Valadares Filho, E. Detmann, M. L. Chizzotti, F. C. Rodrigues, D. Zanetti, and M. G. Machado. 2013b (submitted). Body weight adjustments of pregnant and non-pregnant beef cows. Journal of Animal Science.

Gionbelli, M. P., M. S. Duarte, S. C. Valadares Filho, H. C. Freetly, E. Detmann, D. F. T. Sahtler, and K. E. Hales. 2013a (submitted). Growth and development of cow, udder and gravid uterus of nellore cows during pregnancy. Journal of Animal Science.

Gionbelli, M. P., M. S. Duarte, S. C. Valadares Filho, E. Detmann, M. L. Chizzotti, F. C. Rodrigues, D. Zanetti, and M. G. Machado. 2013b (submitted). Body weight adjustments of pregnant and non-pregnant beef cows. Journal of Animal Science.

Gionbelli, M. P., M. S. Duarte, S. C. Valadares Filho, E. Detmann, M. L. Chizzotti, F. C. Rodrigues, D. Zanetti, and M. G. Machado. 2013c (submitted). The effect of pregnancy and feeding level on voluntary intake, digestion and microbial n production in nellore cows. Journal of Animal Science.

Hammond, J. 1947. Animal breeding in relation to nutrition and environmental conditions. Biological Reviews 22: 195-213.

INRA. 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux. Valeurs des aliments. Editions Quae, Versailles, France.

Johnson, K. A., M. Huyler, H. H. Westberg, B. K. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a sf6 tracer technique. Environmental Science and Technology 28: 359-362.

Marcondes, M. I., M. P. Gionbelli, S. C. Valadares Filho, M. L. Chizzotti, and M. F. Paulino. 2010. Protein requirements of zebu beef cattle. In: S. C. Valadares Filho,

M. I. Marcondes, M. L. Chizzotti and P. V. Paulino (eds.) Nutrient requirements of zebu beef cattle - br-corte No. 2. p 107-126. Suprema Gráfica e Editora, Viçosa.

Mayer, D. G., M. A. Stuart, and A. J. Swain. 1994. Regression of real-world data on model output: An appropriate overall test of validity. Agricultural Systems 45: 93-104.

McCGraham, N. 1964. Energy exchanges of pregnant and lactating ewes. Australian Journal of Agricultural Research 15: 127-141.

Motulsky, H., and A. Christopoulos. 2003. Fitting models to biological data using linear and nonlinear regression. GraphPad Software Inc., San Diego, CA.

NRC – National Research Council. 2000. Nutrient requirements of beef cattle. updated 7th ed. National Academy Press, Washington, DC.

NRC. 1981. Nutritional energetics of domestic animals and glossary of energy terms. Natl. Acad. Press, Washington, DC.

Pinares-Patiño, C. S., K. R. Lassey, R. J. Martin, G. Molano, M. Fernandez, S. MacLean, E. Sandoval, D. Luo, and H. Clark. 2011. Assessment of the sulphur hexafluoride (sf6) tracer technique using respiration chambers for estimation of methane emissions from sheep. Animal Feed Science and Technology 166: 201-209.

Ramin, M., and P. Huhtanen. 2013. Development of equations for predicting methane emissions from ruminants. Journal of Dairy Science 96: 2476-2493.

Ritchie, R. W. 1995. The optimum cow – what criteria must she meet?. In: RESEARCH SYMPOSIUM AND ANNUAL MEETING, 27, Kansas City. p.126-145.

Tedeschi, L. O. 2006. Assessment of the adequacy of mathematical models. Agricultural Systems 89: 225-247.

Theil, H. 1961. Economic forecasts and policy. In: R. Strotz, J. Tinbergen, P. J. Verdoorn and H. J. Witteveen (eds.) Contributions to economic analysis. p 6-48. North-Holland Publishing Company, Amsterdam.

Table 1. Ingredients and chemical composition of the diet

Item	Silage	Concentrate	Diet
Ingredient , % of DM			
Corn Silage	100.0	-	84.3
Ground corn	-	54.6	8.5
Soybean meal	-	33.0	5.1
Urea	-	7.3	1.2
Sodium chloride	-	2.1	0.37
Ammonium sulfate	-	1.5	0.25
Dicalcium phosphate	-	1.3	0.23
Microminerals mixture[1]	-	0.17	0.028
Analyzed composition[2], %			
DM	28.0	89.2	37.6
OM	94.7	92.8	94.4
CP	7.8	44.1	13.5
EE	2.9	2.5	2.8
NDF_{ap}	45.8	8.2	39.9
iNDF	20.8	0.65	17.6
NDIN	38.2	7.0	11.4
NFC	38.2	53.0	40.6
TDN	-	-	66.6
GE (Mcal/kg)	3.82	3.49	3.77

[1]Zinc sulfate (56.3 %), manganese sulfate (26.2 %), copper sulfate (16.8 %), potassium iodate (0.37 %), cobalt sulfate (0.23 %) and sodium selenite (0.10 %).
[2]NDF_{ap} = neutral detergent fiber corrected to ash and protein, iNDF = indigestible neutral detergent fiber and NFC = non fibrous carbohydrates.

Table 2. Partitioning of nutritional requirements by the factorial method and description of the abbreviations used in this study

Recovered	***Net***	***Metabolizable***	***Digestible***
	Maintenance		
-	$NE_m = f(EBW^{0.75})$	$ME_m = f(EBW^{0.75})$ $ME_m = NE_m / k_m$	
	$NP_m = \beta_0$ of $(RP_{ct}/d)=\beta_0+\beta_1\times MPI$	$MP_m = \beta_0$ of $MPI=\beta_0+\beta_1\times rEBG$ $MP_m = NP_m / z_m$	$DE = TME / q$ $q = (GE-UE-EE)/GE$ $TDN = DE / 4.409$
	Pregnancy		
$RE_p = f(DIP)$	NE_p = first derivative of RE_p	$ME_p = NE_p / k_c$ $k_{c1} = NE_p / (MEI – ME_m – ME_g)$ $k_{c2} = (1/\beta_2)$ of $MEI=\beta_0+\beta_1\times RE_{ct}+\beta_2\times RE_p$	
$RN_p = f(DIP)$	NP_p = first derivative of RN_p	$MP_p = NP_p / z_c$ $z_{c1} = NP_p / (MPI – MP_m – MP_g)$ $z_{c2} = (1/\beta_2)$ of $MPI=\beta_0+\beta_1\times RP_{ct}+\beta_2\times RP_p$	
	Weight gain		
$RE_{ct} = f(EBW_{np})$	NE_g = first derivative of RE_{ct} $NE_g = f(EBW_{np}^{0.75}, rEBG)$	$ME_g = NE_g / k_g$ $k_g = \beta_1$ of $(RE_{ct}/d)=\beta_0+\beta_1\times MEI$	$RDP = 1.11\times Pmic$
$RN_{ct} = f(EBW_{np})$	NP_g = first derivative of $RN_{ct}\times 6.25$ $NP_g = f(EBW_{np}^{0.75}, rEBG)$ $NP_g = f(NE_g, rEBG)$	$MP_g = NP_g / z_g$ $z_g = \beta_1$ of $(RP_{ct}/d)=\beta_0+\beta_1\times MPI$	$RUP = ((TMP – (Pmic\times 0.64))/0.8$ $CP = RDP + RUP$
	Total		
$TRE = RE_p + RE_{ct}$	-	$TME = ME_m + ME_p + ME_g$	
$TRN = RN_p + RN_{ct}$	-	$TMP = MP_m + MP_p + MP_g$	

Abbreviations: CP = crude protein, DE = digestible energy, DIP = days in pregnancy, EBW = empty body weight, EBW_{np} = empty body weight in a non-pregnant condition, EE = methane energy, GE = gross energy, k_c = partial efficiency of use of metabolizable energy for pregnancy, k_g = partial efficiency of use of metabolizable energy for gain, k_m = partial efficiency of use of metabolizable energy for maintenance, ME_g = metabolizable energy for gain, MEI = metabolizable energy intake, ME_m = metabolizable energy for maintenance, ME_p = metabolizable energy for pregnancy, MP_g = metabolizable protein for gain, MPI = metabolizable protein intake, MP_m = metabolizable protein for maintenance, MP_p = metabolizable protein for pregnancy, NE_g = net energy for gain, NE_m = net energy for maintenance, NE_p = net energy for pregnancy, NP_g = net protein for gain, NP_m = net protein for maintenance, NP_p = net protein for pregnancy, Pmic = microbial protein, q = metabolizability of energy, RDP = ruminal degradable protein, rEBG = real empty body gain, RE_{ct} = recovered energy in cow body tissues, RE_p = recovered energy in pregnant compound, RN_{ct} = recovered N in cow body tissues, RN_p = recovered N in pregnant compound, RP_{ct} = recovered protein in cow body tissues, RUP = ruminal undegradable protein, TDN = total digestible nutrients, TME = total metabolizable energy, TMP = total metabolizable protein, TRE = total recovered energy, TRN = total recovered N, UE = urinary energy, z_c = partial efficiency of use of metabolizable protein for pregnancy, z_g = partial efficiency of use of metabolizable protein for gain and z_m = partial efficiency of use of protein for maintenance

Table 3. Methane production models[1]

Equations[2]	Statistics			P-value	Equation number
	n	RMSE	r^2		
Methane, L/d = 70.7 ± 24.2** + 0.00385 ± 0.001** × GEI	15	21.6	0.478	0.002	[36]
Methane, L/d = 54.5 ± 29.8* + 0.00774 ± 0.003** × DEI	15	21.6	0.476	0.002	[37]
Methane, L/d = 74.6 ± 21.7** + 0.0128 ± 0.004*** × DMI	15	21.7	0.472	<0.001	[38]
Methane, g/d = 51.2 ± 17.5** + 0.00279 ± 0.001** × GEI	15	15.6	0.479	0.002	[39]
Methane, g/d = 39.5 ± 21.6* + 0.00561 ± 0.002** × DEI	15	15.6	0.476	0.003	[40]
Methane, g/d = 54.1 ± 15.7** + 0.00926 ± 0.003*** × DMI	15	15.7	0.472	<0.001	[41]
Methane, kcal/d = 626 ± 214** + 0.0341 ± 0.01** × GEI	15	191	0.478	0.002	[42]
Methane, kcal/d = 483 ± 264* + 0.0685 ± 0.02** × DEI	15	192	0.476	0.002	[43]
Methane, kcal/d = 660 ± 192** + 0.113 ± 0.03*** × DMI	15	192	0.472	<0.001	[44]
Methane, kcal/100kcalGEI = 9.65 ± 1.53*** – 0.00014 ± 0.00008* × GEI	15	1.15	0.391	0.110	[45]
Methane, kcal/100kcalDEI = 14.7 ± 2.74*** – 0.0003 ± 0.0002* × DEI	15	1.81	0.212	0.025	[46]

[1]GEI = gross energy intake, kcal/d; DEI = digestible energy intake, kcal/d; DMI = dry matter intake, g/d; Values within parentheses are SE of the parameter estimate; *P<0.10; **P<0.05; P<0.01; RMSE = Root-mean-square error.

[2]Estimations are safe only between 9,327 and 33,688 kcal/d of GEI, 6,733 and 18,850 kcal/d of DEI and 2,501 and 9,828 g/d of DMI.

Table 4. Example of metabolizable energy requirements for pregnant and non-pregnant Nellore cows at different situations

Gestation, d	BW, kg	rEBG, kg/d	SBW, kg	EBW_{np}, kg	PREG, kg	NE_p[a], Mcal/d	NE_p[b], Mcal/d	ME_p[a], Mcal/d	ME_p[b], Mcal/d	ME_g, Mcal/d	ME_m, Mcal/d	TME[a], Mcal/d	TME[b], Mcal/d
0	400	-	387.7	351.3	-	-	-	-	-	-	8.40	8.40	8.40
0	550	-	538.3	489.6	-	-	-	-	-	-	10.77	10.77	10.77
250	400	-	387.7	317.9	36.5	0.41	0.40	3.41	3.35	-	8.45	11.87	11.81
250	550	-	538.3	456.1	36.5	0.41	0.40	3.41	3.35	-	10.82	14.23	14.17
0	400	0.5	387.7	351.3	-	-	-	-	-	9.32	8.40	17.72	17.72
0	550	0.5	538.3	489.6	-	-	-	-	-	11.96	10.77	22.73	22.73
250	400	0.5	387.7	317.9	36.5	0.41	0.40	3.41	3.35	8.65	8.45	20.52	20.46
250	550	0.5	538.3	456.1	36.5	0.41	0.40	3.41	3.35	11.34	10.82	25.58	25.52

[a]Using logistic function to estimate NE_p.
[b]Using allometric function to estimate NE_p.

Figure captions

Figure 1. Relationship between energy and nitrogen in cow tissues and pregnant compound of pregnant and non-pregnant Nellore cows

Figure 2. Net energy requirements for pregnancy estimated in this study and by NRC (2000) for a calf weight at birth of 28 kg

Figure 3. Net energy requirements for pregnancy estimated in this study in proportion (%) of estimated by NRC (2000) for *B. taurus* cattle for an calf weight at birth of 28 kg

Figure 4. Illustration of the partitioning of total metabolizable energy requirements for a 450 kg BW cow with a rEBG of 0.3 kg/d. Estimated calf birth weight = 30 kg.

Figure 1

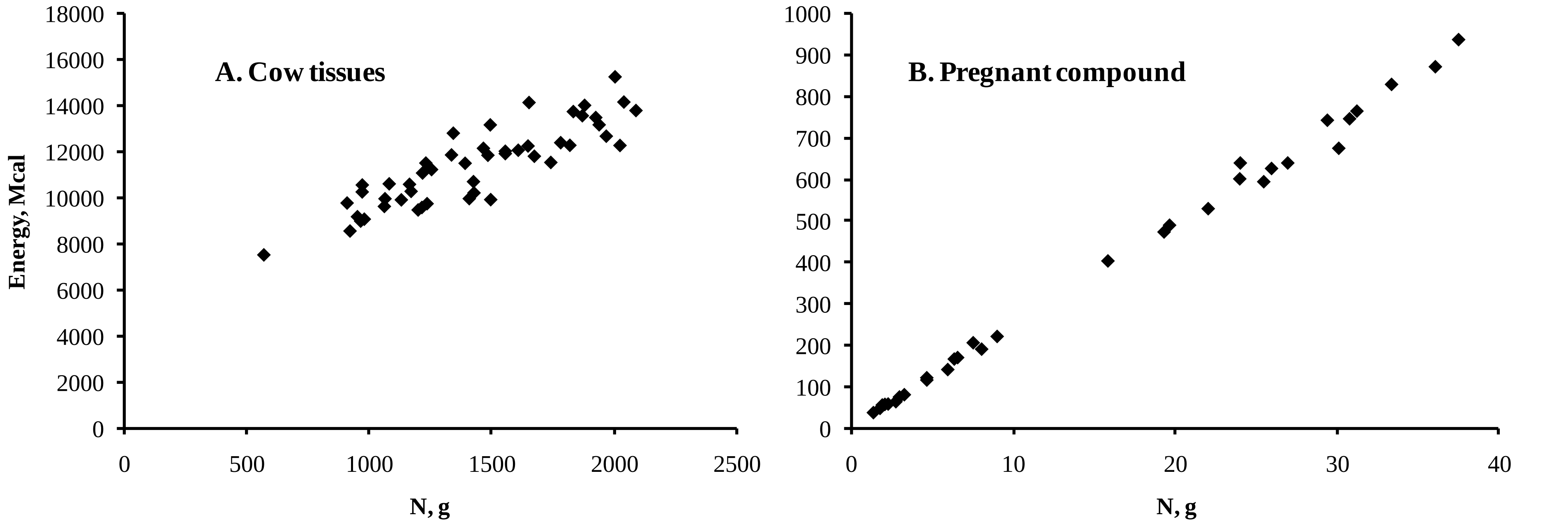

A. Cow tissues
Energy, Mcal
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
500
1000
1500
2000
2500
N, g
B. Pregnant compound
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
N, g

Figure 2

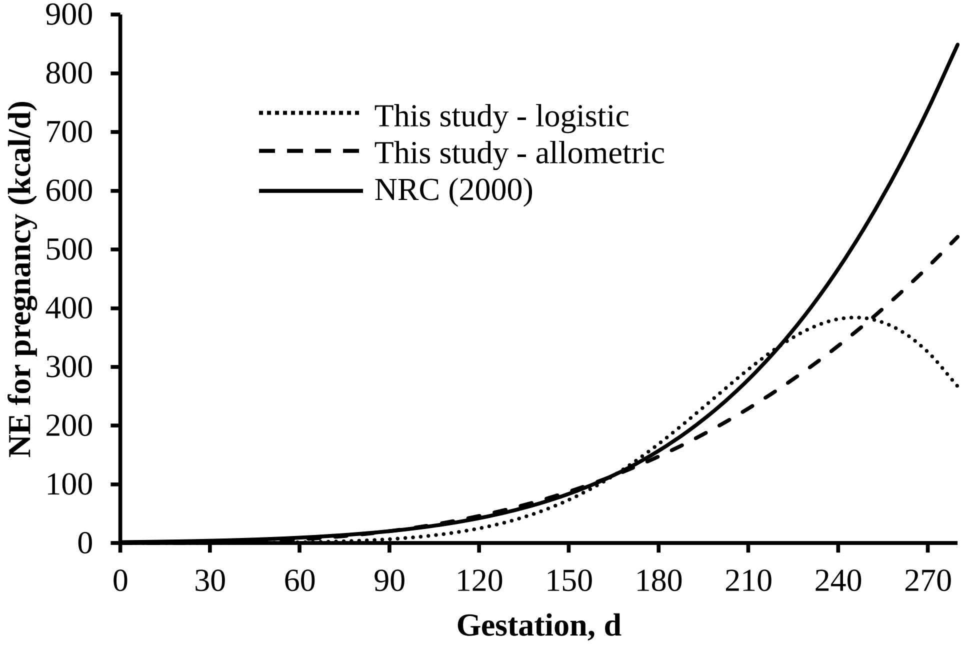
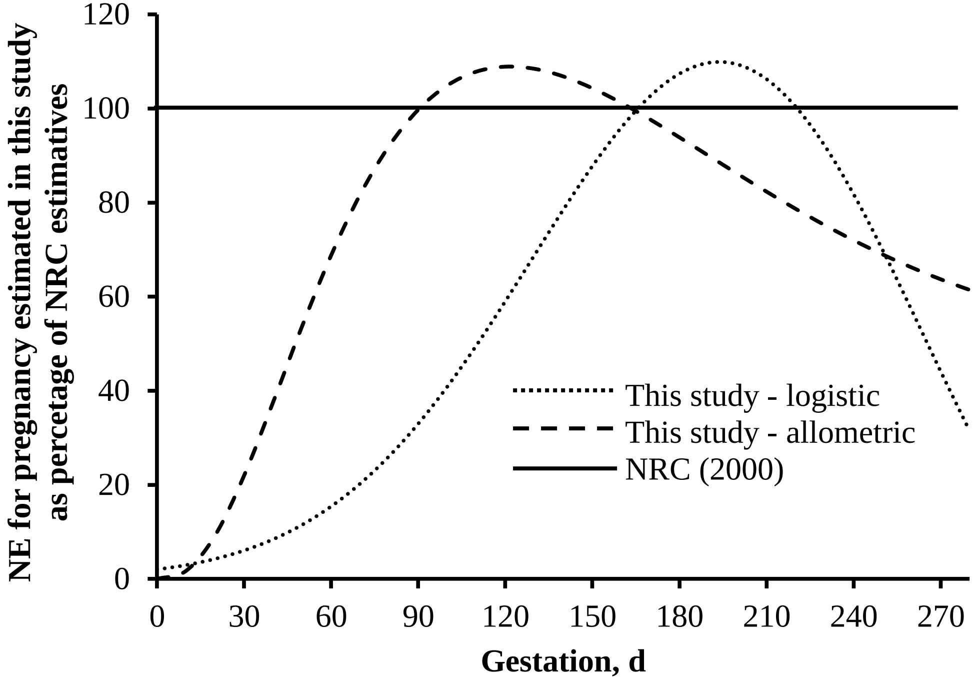
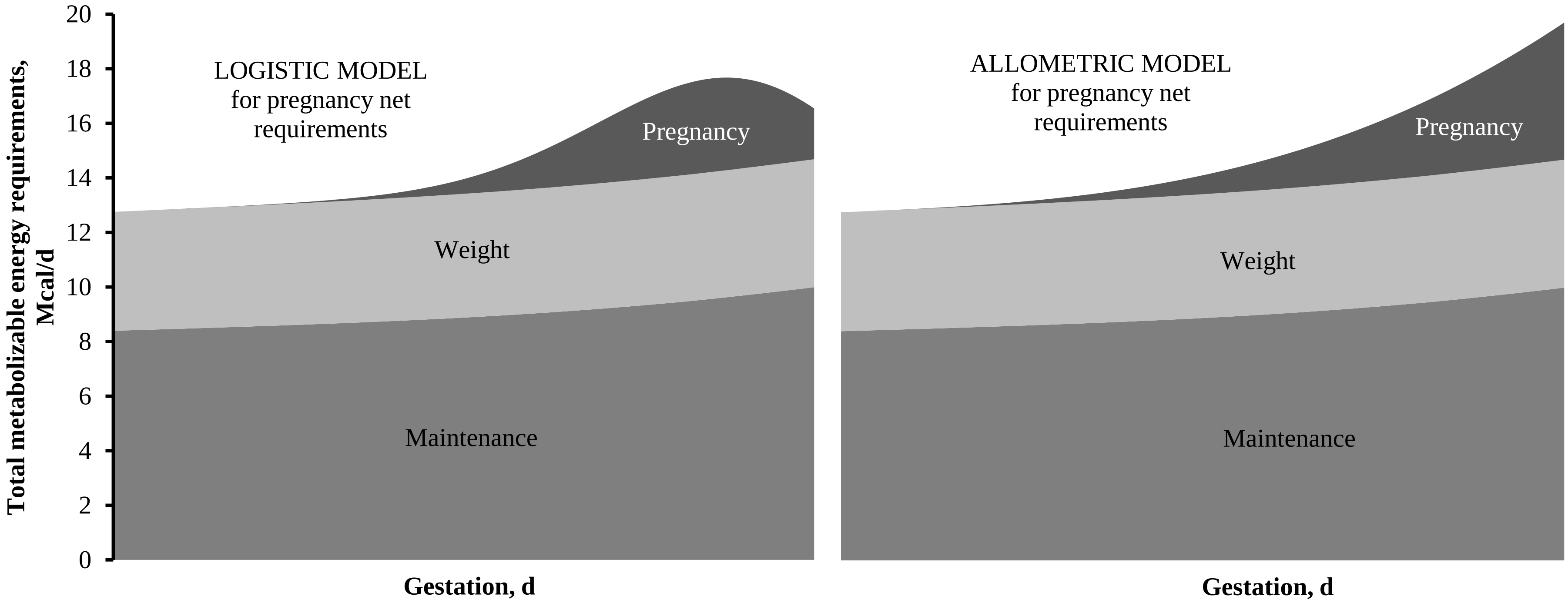

900
800
700
600
500
400
300
200
100
0
NE for pregnancy (kcal/d)
This study - logistic
This study - allometric
NRC (2000)
0
30
60
90
120
150
180
210
240
270
Gestation, d

Figure 3



NE for pregnancy estimated in this study
as percetage of NRC estimatives
120
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
270
Gestation, d
This study - logistic
This study - allometric
NRC (2000)

Figure 4



20
18
16
14
12
10
8
6
4
2
0
Total metabolizable energy requirements, Mcal/d
LOGISTIC MODEL
for pregnancy net
requirements
Pregnancy
Weight
Maintenance
Gestation, d
ALLOMETRIC MODEL
for pregnancy net
requirements
Pregnancy
Weight
Maintenance
Gestation, d

APPENDIX

Data presented in this Appendix are public and available for use in collaborative studies citing the correct source. Additional information may be obtained by contacting the following emails: mateus@zootecnista.com.br or scvfilho@ufv.br

Table 1. # = cow code, Treat = treatment (High or Low feeding level, B = baseline group), DIP = days in pregnancy at slaughter, iBW = initial body weight (kg), iSBW = initial shrunk body weight (kg), iEBW = initial empty body weight (kg), fBW = final body weight (kg), fSBW = final shrunk body weight (kg), fEBW = final empty body weight (kg), DEXP = days in experiment (feeding control), iBCS = initial body condition score, fBCS = final body condition score. All remaining variables are in kg.

#	Treat	DIP	iBW	iSBW	iEBW	fBW	fSBW	fEBW	DEXP	iBCS	fBCS	Non-carcass	Carcass	Udder	Gravid Uterus	Uterus + Ovaries	Fetus	Placenta	Fluids
1	High	240	515,0	495,0	446,4	656,0	647,0	602,0	204	5,0	8,0	182,25	384,15	4,77	30,87	6,19	17,84	1,14	5,70
2	High	0	446,5	435,0	391,6	542,0	535,5	496,2	122	4,5	6,0	169,52	320,00	5,91	-	0,73	-	-	-
3	High	189	486,0	470,0	423,5	616,0	605,5	550,4	145	5,0	8,0	178,52	353,75	3,67	14,42	3,49	6,66	1,21	3,06
4	B	0	596,0	588,0	507,2	596,0	588,0	507,2	0	4,5	4,5	164,11	338,35	4,17	-	0,62	-	-	-
5	Low	139	444,8	432,5	389,4	-	477,5	442,0	122	4,5	4,5	135,81	289,30	4,51	12,33	3,30	1,61	0,48	6,95
6	Low	234	450,0	439,0	395,3	-	480,0	432,8	185	4,5	5,0	122,11	266,55	3,65	40,50	8,96	21,51	1,24	8,79
7	Low	0	520,5	508,0	458,2	-	531,5	478,7	185	4,0	4,5	158,10	316,10	3,83	-	0,71	-	-	-
8	High	269	503,0	486,0	438,1	625,5	623,5	583,6	204	5,0	6,5	172,91	357,15	5,92	47,62	8,69	27,51	1,79	9,63
9	Low	0	513,0	509,0	459,1	-	531,5	484,4	85	8,0	8,0	154,62	323,60	5,76	-	0,42	-	-	-
10	High	265	529,0	516,0	465,5	710,0	701,0	653,6	216	6,5	8,0	203,88	382,15	9,32	58,22	10,29	33,27	1,61	13,04
11	Low	138	612,1	601,0	543,2	-	641,0	572,1	122	5,0	5,5	161,63	393,65	5,57	11,28	3,01	1,78	0,41	6,08
12	Low	269	460,0	449,0	404,4	-	492,5	456,9	216	4,5	5,0	137,15	278,25	3,59	37,93	6,46	21,70	1,25	8,53
13	High	189	393,0	377,0	338,8	452,5	447,5	407,1	143	4,0	5,5	132,31	256,95	3,69	14,14	3,15	5,51	1,02	4,45
14	Low	272	424,0	414,0	372,5	-	466,5	434,0	227	3,5	4,0	123,43	260,75	4,03	45,84	8,34	27,59	1,28	8,63
15	Low	135	426,0	417,0	375,2	-	434,0	394,1	78	3,5	4,0	124,00	259,20	3,48	7,46	1,94	1,47	0,44	3,61
16	High	191	418,0	407,0	366,1	548,0	545,5	497,3	145	5,0	7,0	163,85	311,95	4,45	17,01	3,59	7,70	1,09	4,63
17	Low	188	471,0	460,0	414,4	-	453,5	419,1	126	4,0	4,5	133,72	270,00	5,15	10,23	2,17	5,30	0,39	2,37
18	Low	266	388,0	372,0	334,3	-	457,0	423,8	229	3,5	5,5	123,86	252,25	4,12	43,57	7,82	25,67	1,25	8,82
19	High	236	535,0	522,0	471,0	692,0	688,0	624,7	185	6,0	7,5	187,16	394,80	5,15	37,56	6,85	20,10	1,64	8,97
20	High	140	576,0	565,0	510,3	623,5	618,5	564,2	90	7,0	7,5	194,54	355,95	3,93	9,81	2,84	2,02	0,71	4,24
21	High	0	504,0	484,0	436,3	600,0	588,0	546,1	145	4,0	6,0	175,03	363,30	7,06	-	0,76	-	-	-

22	Low	240	551,0	535,0	482,9	-	610,5	574,0	196	4,0	6,0	148,36	379,00	4,33	42,33	9,51	21,94	1,41	9,46
23	Low	0	563,0	550,0	496,6	-	622,0	562,6	216	5,0	6,0	169,53	387,22	5,12	-	0,75	-	-	-
24	Low	269	397,3	385,0	346,1	-	478,5	452,6	262	3,5	5,0	131,81	274,50	5,81	40,43	7,97	22,69	1,25	8,52
26	High	268	373,0	363,5	326,6	583,5	573,5	538,3	215	4,0	7,5	147,84	325,70	6,87	57,91	10,38	31,60	1,94	13,99
27	Low	241	337,0	329,0	295,2	-	384,0	361,7	181	3,0	4,0	110,41	222,55	2,47	26,25	5,08	13,99	1,05	6,13
28	Low	190	418,8	406,5	365,7	-	460,0	422,3	153	5,0	5,5	122,19	274,77	4,34	20,98	4,40	8,09	0,91	7,58
29	High	0	327,0	313,5	281,1	373,5	366,5	341,4	85	3,5	4,5	116,09	221,05	3,75	-	0,50	-	-	-
30	B	0	303,0	299,0	269,1	303,0	299,0	269,1	0	3,0	3,0	93,50	173,65	1,66	-	0,33	-	-	-
31	High	134	443,0	430,0	387,1	509,5	490,0	451,1	97	5,0	6,0	128,29	311,80	5,00	6,00	1,60	1,17	0,37	2,87
32	High	0	470,0	450,5	405,8	554,5	546,0	478,8	85	4,5	6,5	161,84	312,15	3,91	-	0,88	-	-	-
33	Low	0	461,0	440,0	396,2	-	454,0	417,4	97	5,0	5,0	128,77	284,35	3,81	-	0,50	-	-	-
34	B	0	455,0	437,0	389,4	455,0	437,0	389,4	0	4,5	4,5	132,50	253,00	3,29	-	0,59	-	-	-
35	High	270	421,0	405,0	364,3	630,5	617,5	577,5	218	4,0	8,0	148,86	377,40	7,84	43,37	6,91	26,53	1,24	8,70
37	Low	192	428,0	412,0	370,7	-	464,0	405,6	162	3,0	4,5	128,01	254,80	4,43	18,36	4,36	7,11	0,74	6,14
38	Low	239	487,0	465,0	419,0	-	537,0	485,1	197	5,5	6,0	134,19	307,70	3,64	39,58	7,06	22,69	1,19	8,64
39	High	191	424,5	414,5	373,0	545,0	536,5	497,4	156	5,0	6,5	151,86	329,10	4,06	12,37	2,48	6,00	0,80	3,08
40	High	240	400,5	380,5	342,0	534,5	526,5	483,0	172	4,5	7,0	146,68	300,64	3,79	31,86	5,09	19,84	1,12	5,80
41	Low	0	439,0	417,0	375,2	-	440,5	397,4	97	5,0	5,5	129,69	263,70	3,63	-	0,40	-	-	-
42	High	240	436,0	417,5	375,7	586,0	572,0	528,2	172	5,0	7,5	154,15	328,05	7,51	38,46	6,98	21,34	1,35	8,79
43	Low	0	365,0	351,0	315,2	-	394,0	352,2	118	2,5	3,5	118,65	229,40	3,70	-	0,48	-	-	-
44	Low	0	405,5	392,5	352,9	-	407,0	357,4	85	3,5	3,5	115,88	237,15	3,82	-	0,59	-	-	-
45	High	0	412,0	400,5	360,2	545,0	539,0	495,4	140	3,5	5,5	155,46	335,70	3,75	-	0,48	-	-	-
46	B	0	461,0	448,0	397,2	461,0	448,0	397,2	0	4,5	4,5	132,73	260,55	3,33	-	0,57	-	-	-
47	B	0	460,0	450,5	396,3	460,0	450,5	396,3	0	4,5	4,5	130,57	262,90	2,41	-	0,47	-	-	-
48	High	130	412,0	402,5	362,0	502,0	499,0	439,0	74	4,0	5,5	150,94	274,20	5,44	8,38	1,88	0,99	0,44	5,07
49	Low	185	432,0	428,0	385,3	-	438,0	387,8	85	4,5	4,5	129,32	244,10	2,68	11,66	3,24	4,25	0,61	3,56
50	Low	134	386,0	377,0	338,8	-	401,5	366,3	99	3,5	4,0	120,28	235,20	2,57	8,22	2,19	1,34	0,43	4,26
51	High	135	405,0	405,0	364,3	516,5	508,5	452,2	85	4,0	5,5	152,57	286,90	4,62	8,07	2,11	1,64	0,50	3,81

Table 2. # = cow code, SFT = subcutaneous fat thickness, DMI = dry matter intake, DUdp = gravid uterus accretion due pregnancy (kg), PREG = pregnant compound (kg), SBWnp = non-pregnant shrunk body weight (kg), EBWnp = non-pregnant empty body weight (kg), aDG = average daily gain (kg/day), aSBG = average shrunk body gain (kg/day), aEBG = average empty body gain (kg/day), tEBG = total empty body gain (kg), rSBG = real shrunk body gain (kg/day) and rEBG = real empty body gain (kg/day).

#	SFT (mm)	Loin area (cm^2)	DMI (kg/day)	DMI (g/kg SBW/day)	GUdp[a]	PREG[a]	SBWnp[a]	EBWnp[a]	aDG	aSBG	aEBG	tEBG	rSBG[a]	rEBG[a]
1	9,70	70,03	8,61	15,08	30,13	30,24	616,76	571,79	0,69	0,75	0,76	155,68	0,60	0,61
2	10,71	61,49	8,11	16,72	-	-	535,50	496,15	0,78	0,82	0,86	104,52	0,82	0,86
3	12,84	68,75	8,44	15,69	13,71	13,71	591,79	536,65	0,90	0,93	0,87	126,82	0,84	0,78
4	5,20	62,04	-	-	-	-	588,00	507,25	-	-	-	-	-	-
5	9,24	63,90	4,07	8,94	11,77	11,77	465,73	430,18	-	0,37	0,43	52,60	0,27	0,33
6	10,33	63,46	4,80	10,44	39,97	39,97	440,03	392,83	-	0,22	0,20	37,52	0,01	-0,01
7	3,66	62,14	5,56	10,69	-	-	531,50	478,74	-	0,13	0,11	20,52	0,13	0,11
8	14,40	68,38	7,36	13,27	46,93	48,91	574,59	534,69	0,60	0,67	0,71	145,45	0,43	0,47
9	12,06	69,12	5,55	10,66	-	-	531,50	484,41	-	0,26	0,30	25,28	0,26	0,30
10	13,45	70,93	8,19	13,47	57,45	59,41	641,59	594,15	0,84	0,86	0,87	188,04	0,58	0,60
11	5,68	65,28	4,90	7,89	10,52	10,52	630,48	561,61	-	0,33	0,24	28,92	0,24	0,15
12	9,78	56,51	4,81	10,21	37,39	38,87	453,63	418,05	-	0,20	0,24	52,52	0,02	0,06
13	6,04	59,53	7,34	17,80	13,62	13,62	433,88	393,47	0,42	0,49	0,48	68,26	0,40	0,38
14	6,24	59,57	4,72	10,72	45,34	46,80	419,70	387,24	-	0,23	0,27	61,54	0,03	0,06
15	3,10	53,51	5,37	12,62	6,95	6,95	427,05	387,18	-	0,22	0,24	18,90	0,13	0,15
16	12,75	80,53	8,27	17,36	16,38	16,38	529,12	480,88	0,90	0,96	0,90	131,13	0,84	0,79
17	5,85	50,66	5,52	12,09	9,70	9,70	443,80	409,40	-	-0,05	0,04	4,67	-0,13	-0,04
18	3,88	57,60	4,47	10,78	43,07	44,29	412,71	379,51	-	0,37	0,39	89,52	0,18	0,20
19	7,09	74,22	8,18	13,53	36,78	36,78	651,22	587,89	0,85	0,90	0,83	153,67	0,70	0,63
20	15,34	82,10	8,06	13,61	9,08	9,08	609,42	555,15	0,53	0,59	0,60	53,94	0,49	0,50
21	6,62	76,02	8,27	15,43	-	-	588,00	546,14	0,66	0,72	0,76	109,83	0,72	0,76
22	5,51	76,70	5,28	9,22	41,65	41,74	568,76	532,27	-	0,39	0,47	91,14	0,17	0,25
23	5,56	89,55	5,05	8,62	-	-	622,00	562,62	-	0,33	0,31	66,04	0,33	0,31
24	6,90	58,62	3,99	9,23	39,91	41,33	437,17	411,22	-	0,36	0,41	106,45	0,20	0,25
26	11,53	61,22	7,44	15,89	57,29	59,04	514,46	479,27	0,98	0,98	0,98	211,76	0,70	0,71
27	3,69	57,66	4,43	12,43	25,82	25,91	358,09	335,77	-	0,30	0,37	66,47	0,16	0,22

28	4,85	58,93	4,65	10,74	20,45	20,45	439,55	401,82	-	0,35	0,37	56,59	0,22	0,24
29	4,98	57,90	6,47	19,04	-	-	366,50	341,39	0,55	0,62	0,71	60,26	0,62	0,71
30	1,80	52,69	-	-	-	-	299,00	269,13	-	-	-	-	-	-
31	10,61	72,00	6,74	14,65	5,42	5,42	484,58	445,67	0,69	0,62	0,66	64,01	0,56	0,60
32	12,54	60,70	8,93	17,93	-	-	546,00	478,79	0,99	1,12	0,86	73,03	1,12	0,86
33	5,49	77,65	5,20	11,63	-	-	454,00	417,43	-	0,14	0,22	21,23	0,14	0,22
34	3,25	50,31	-	-	-	-	437,00	389,37	-	-	-	-	-	-
35	13,81	67,49	7,94	15,54	42,68	44,82	572,68	532,65	0,96	0,97	0,98	213,16	0,77	0,77
37	4,08	52,14	4,63	10,56	17,83	17,83	446,17	387,77	-	0,32	0,22	34,91	0,21	0,11
38	5,77	68,28	4,86	9,71	38,98	39,02	497,98	446,09	-	0,37	0,34	66,12	0,17	0,14
39	12,27	85,58	7,08	14,89	11,74	11,74	524,76	485,65	0,77	0,78	0,80	124,43	0,71	0,72
40	8,70	63,90	6,72	14,82	31,27	31,35	495,15	451,61	0,78	0,85	0,82	140,95	0,67	0,64
41	5,12	64,08	4,87	11,35	-	-	440,50	397,42	-	0,24	0,23	22,19	0,24	0,23
42	8,37	75,74	7,28	14,71	37,82	37,92	534,08	490,25	0,87	0,90	0,89	152,47	0,68	0,67
43	2,21	52,52	5,18	13,91	-	-	394,00	352,22	-	0,36	0,31	37,03	0,36	0,31
44	2,18	53,38	4,90	12,25	-	-	407,00	357,43	-	0,17	0,05	4,50	0,17	0,05
45	7,10	71,21	8,12	17,29	-	-	539,00	495,39	0,95	0,99	0,97	135,18	0,99	0,97
46	6,70	48,53	-	-	-	-	448,00	397,18	-	-	-	-	-	-
47	4,41	59,57	-	-	-	-	450,50	396,34	-	-	-	-	-	-
48	5,06	70,42	9,24	20,50	7,79	7,79	491,21	431,16	1,22	1,30	1,04	76,92	1,20	0,93
49	4,70	56,59	4,76	10,99	11,15	11,15	426,85	376,61	-	0,12	0,03	2,50	-0,01	-0,10
50	3,18	51,96	5,43	13,96	7,75	7,75	393,75	358,52	-	0,25	0,28	27,44	0,17	0,20
51	5,72	55,91	8,17	17,89	7,47	7,47	501,03	444,69	1,31	1,22	1,03	87,85	1,13	0,95

[a]See chapter 2.

Table 3. All variables measured at slaughter. # = cow code, N-NC = non-carcass nitrogen (kg), N-C = carcass nitrogen (kg), N-Ut = uterus nitrogen (kg), N-Ud = udder nitrogen (kg), EE-NC = non-carcass ether extract (kg), EE-C = carcass ether extract (kg), EE-Ut = uterus ether extract (kg), EE-Ud = udder ether extract (kg), A-NC = non-carcass ash (kg), A-C = carcass ash (kg), A-Ut = uterus ash (kg), A-Ud = udder ash (kg), W-NC = non-carcass water (kg), W-C = carcass water (kg), W-Ut = uterus water (kg) and W-Ud = udder water (kg).

#	N-NC	N-C	N-Ut	N-Ud	EE-NC	EE-C	EE-Ut	EE-Ud	A-NC	A-C	A-Ut	A-Ud	W-NC	W-C	W-Ut	W-Ud
1	4,55	9,13	0,49	0,085	57,35	108,74	0,64	3,19	5,78	15,95	0,62	0,024	91,05	190,86	26,31	0,83
2	4,33	7,95	0,02	0,075	51,53	87,32	0,02	4,55	5,24	10,31	0,01	0,022	86,03	167,97	0,59	0,33
3	5,38	8,54	0,19	0,064	40,54	102,93	0,18	2,28	4,75	15,03	0,22	0,019	99,87	178,46	12,73	0,71
4	4,51	8,50	0,02	0,121	35,17	73,90	0,03	2,45	6,32	16,95	0,01	0,023	92,34	185,32	0,48	0,36
5	4,16	6,43	0,09	0,083	32,10	93,59	0,09	2,97	3,46	12,27	0,11	0,032	74,14	139,85	11,50	0,92
6	3,22	6,24	0,61	0,090	25,34	70,00	0,91	1,70	2,53	18,05	0,89	0,026	73,08	134,85	34,63	1,24
7	4,42	8,26	0,02	0,091	35,89	65,49	0,02	2,04	4,42	16,80	0,01	0,027	90,62	175,16	0,57	0,97
8	4,57	7,55	0,75	0,124	57,56	118,84	1,11	4,10	4,20	19,34	1,15	0,034	83,09	165,63	40,18	0,90
9	4,20	7,70	0,01	0,098	40,24	77,85	0,01	4,47	5,22	17,82	0,00	0,027	82,39	170,39	0,32	0,45
10	5,28	9,78	0,89	0,170	58,13	96,47	1,26	4,70	5,64	12,44	1,32	0,058	107,95	204,20	49,52	2,54
11	4,31	9,04	0,08	0,098	37,57	116,79	0,08	4,26	4,84	19,76	0,11	0,022	91,11	191,58	10,49	0,43
12	3,13	6,68	0,65	0,080	40,72	80,56	0,58	1,64	5,35	10,75	0,84	0,027	71,35	140,33	32,22	1,35
13	3,49	6,69	0,18	0,066	28,78	52,26	0,14	1,94	4,64	15,04	0,22	0,022	76,07	141,56	12,54	0,95
14	3,21	6,40	0,84	0,111	26,95	67,80	1,05	1,60	5,45	12,43	1,31	0,031	69,83	136,62	38,03	1,61
15	3,18	6,21	0,06	0,051	30,72	61,16	0,04	2,39	4,39	13,41	0,08	0,014	68,77	137,28	6,94	0,46
16	4,26	7,66	0,21	0,109	48,35	86,66	0,20	2,86	6,17	9,46	0,26	0,027	82,30	161,69	15,05	0,77
17	3,63	6,45	0,13	0,104	34,88	72,98	0,14	3,91	4,05	15,30	0,17	0,029	71,95	135,01	9,05	0,50
18	3,59	5,90	0,77	0,109	21,69	60,69	0,89	1,82	5,14	9,32	1,30	0,029	73,98	141,53	36,21	1,36
19	4,15	9,86	0,55	0,114	65,09	105,27	0,64	3,14	3,85	17,11	0,74	0,043	91,91	204,16	32,36	1,01
20	5,19	8,82	0,09	0,093	50,66	82,12	0,07	2,27	5,95	17,50	0,09	0,028	105,92	193,04	8,98	1,00
21	4,88	8,15	0,02	0,103	49,66	111,16	0,02	5,39	5,31	14,08	0,01	0,036	89,82	179,11	0,61	0,75
22	4,36	9,09	0,65	0,088	36,40	114,53	0,81	2,36	4,21	20,95	0,99	0,028	80,62	180,94	36,02	1,19
23	4,10	9,53	0,02	0,081	52,79	89,39	0,02	4,05	9,14	14,79	0,01	0,023	82,47	207,83	0,60	0,32
24	4,68	6,67	0,68	0,132	13,73	72,38	1,11	2,19	4,15	12,29	1,13	0,045	84,40	142,85	33,55	2,46
26	4,05	7,29	0,95	0,171	39,73	96,80	1,13	3,66	3,84	17,95	1,43	0,042	78,63	159,71	49,01	2,01
27	2,97	5,51	0,41	0,048	21,00	48,46	0,55	1,34	2,62	7,80	0,54	0,015	67,93	127,05	22,42	0,67

28	3,20	6,65	0,23	0,086	32,07	77,34	0,27	2,84	5,50	13,55	0,29	0,030	64,82	137,44	18,83	0,85
29	3,17	5,72	0,01	0,069	25,73	43,73	0,01	2,74	4,23	9,90	0,00	0,019	65,86	126,69	0,41	0,46
30	2,87	4,59	0,01	0,037	10,11	24,84	0,01	0,83	3,90	9,09	0,00	0,011	60,71	106,66	0,25	0,50
31	4,67	7,08	0,05	0,067	28,11	90,01	0,03	3,47	4,00	13,70	0,06	0,016	71,12	157,40	5,56	0,66
32	3,86	7,93	0,03	0,079	47,40	77,12	0,03	2,96	7,22	16,40	0,01	0,019	83,12	162,89	0,67	0,39
33	3,61	7,37	0,01	0,063	29,38	58,07	0,01	2,91	3,99	12,23	0,00	0,015	72,51	161,79	0,40	0,26
34	3,62	6,53	0,01	0,073	25,64	38,17	0,02	2,16	4,78	13,56	0,00	0,018	78,09	154,21	0,50	0,62
35	4,22	8,24	0,76	0,188	30,58	125,26	0,94	3,81	3,70	12,89	0,89	0,069	87,93	179,82	36,26	2,67
37	3,72	6,77	0,22	0,089	21,69	52,58	0,18	3,00	3,28	13,43	0,25	0,021	79,49	142,17	16,49	0,76
38	4,10	7,64	0,64	0,102	20,94	77,56	0,80	1,39	3,85	12,42	0,92	0,033	82,73	165,30	33,56	1,51
39	3,94	7,75	0,16	0,082	37,42	97,33	0,19	2,81	5,14	14,48	0,19	0,022	82,16	164,03	10,90	0,68
40	4,07	7,97	0,50	0,080	37,37	71,11	0,59	2,09	3,82	13,95	0,73	0,020	80,83	161,28	27,17	0,85
41	3,47	7,02	0,01	0,072	35,34	55,41	0,01	2,87	5,53	14,24	0,00	0,017	67,51	144,55	0,32	0,26
42	3,81	8,32	0,61	0,115	51,56	94,14	0,80	5,29	2,33	16,66	0,77	0,042	76,41	160,98	32,63	0,90
43	3,50	6,16	0,01	0,085	20,52	38,84	0,02	2,55	4,91	11,86	0,00	0,017	70,47	133,93	0,38	0,58
44	3,16	5,94	0,01	0,054	29,97	44,14	0,01	2,37	4,52	14,47	0,00	0,017	62,04	138,19	0,49	0,50
45	4,00	8,16	0,01	0,061	38,24	90,35	0,02	2,96	4,97	11,88	0,00	0,017	85,05	177,47	0,38	0,30
46	3,85	5,97	0,01	0,057	30,63	61,00	0,01	2,01	5,62	14,17	0,00	0,012	72,68	141,11	0,48	0,75
47	3,80	6,68	0,01	0,048	23,43	44,93	0,01	1,28	5,63	12,64	0,00	0,010	76,64	156,29	0,37	0,65
48	4,47	6,61	0,07	0,113	33,21	57,21	0,05	4,01	5,57	15,24	0,09	0,031	83,89	154,27	7,76	0,56
49	3,80	6,07	0,13	0,057	23,34	55,26	0,11	1,55	3,78	17,57	0,14	0,014	78,72	127,48	10,49	0,65
50	3,24	5,74	0,06	0,061	23,31	46,09	0,04	1,63	3,84	9,75	0,08	0,014	72,65	137,86	7,66	0,53
51	4,19	7,17	0,07	0,110	37,43	67,38	0,05	3,00	7,00	15,44	0,08	0,025	82,01	153,54	7,46	0,76

Table 4. # = cow code, EN-NC = non-carcass energy (Mcal), EN-C = carcass energy (Mcal), EN-Ut = uterus energy (Mcal), EN-Ud = udder energy (Mcal), CB-RE = cow's body recovered energy (Mcal), CB-RN = cow's body recovered nitrogen (g), PREG-RE = pregnant compound recovered energy (Mcal), PREG-RN = pregnant compound recovered energy (Mcal), MEI = metabolizable energy intake (Mcal/day), RET = retained energy (Mcal/day), REP = retained energy in pregnant compound (Mcal/day), RECB = retained energy in cow's body (Mcal/day), HP = heat production (Mcal/day).

#	EN-NC	EN-C	EN-Ut	EN-Ud	CB-RE	CB-RN	PREG-RE	PREG-RN	MEI	RET	REP	RECB	HP
1	665,4	1390	20,00	33,60	2090	13776	19,30	472,7	20,47	3,95	0,095	3,86	16,62
2	703,5	1033	0,70	44,82	1782	12377	-	-	19,53	5,54	0,000	5,54	13,99
3	574,9	1281	7,22	22,80	1880	14001	6,54	168,8	20,03	4,51	0,045	4,46	15,57
4	456,6	1010	0,73	26,56	1494	13151	-	-	-	-	-	-	-
5	368,1	1025	3,76	31,22	1425	10684	3,25	78,9	10,26	2,73	0,027	2,71	7,55
6	358,4	835	24,46	19,99	1214	9561	23,97	601,3	12,14	0,77	0,130	0,64	11,50
7	443,5	876	0,68	22,54	1342	12790	-	-	13,90	0,12	0,000	0,12	13,78
8	575,1	1406	30,04	42,99	2024	12257	29,39	743,1	17,31	3,88	0,144	3,73	13,57
9	489,9	1019	0,50	45,91	1555	12007	-	-	13,80	0,97	0,000	0,97	12,82
10	658,6	1295	36,80	49,63	2004	15240	36,06	872,5	19,41	2,90	0,167	2,73	16,68
11	470,0	1412	3,45	42,77	1925	13471	2,73	62,0	12,36	1,99	0,022	1,96	10,40
12	498,1	978	24,51	18,64	1495	9901	24,01	639,6	12,20	1,82	0,111	1,71	10,49
13	370,2	778	6,81	20,49	1170	10262	6,34	165,2	17,31	1,92	0,044	1,88	15,43
14	373,3	843	33,81	18,61	1235	9726	33,35	829,8	11,97	1,33	0,147	1,18	10,78
15	368,3	806	2,21	23,41	1198	9453	1,75	45,9	13,45	2,47	0,022	2,45	11,00
16	546,5	1029	8,62	31,80	1608	12050	8,03	189,2	19,65	4,07	0,055	4,02	15,63
17	423,9	961	5,13	41,49	1427	10196	4,64	114,4	13,91	2,15	0,037	2,11	11,80
18	332,1	708	31,66	19,59	1060	9608	31,21	765,2	11,32	1,09	0,136	0,95	10,37
19	737,5	1268	22,78	33,72	2040	14145	22,03	529,1	19,39	3,41	0,119	3,29	16,10
20	585,2	1042	3,65	24,26	1652	14123	2,95	73,5	19,12	0,15	0,033	0,11	19,01
21	553,8	1330	0,73	54,99	1939	13155	-	-	20,00	4,81	0,000	4,81	15,19
22	469,6	1375	27,58	25,28	1870	13550	26,94	639,7	13,38	2,63	0,137	2,49	10,89
23	632,2	1160	0,71	40,20	1833	13729	-	-	12,63	1,50	0,000	1,50	11,13
24	298,6	906	30,57	25,03	1230	11492	30,09	675,2	10,06	1,44	0,115	1,33	8,74
26	519,0	1181	38,07	40,64	1741	11519	37,50	938,2	17,54	4,42	0,174	4,24	13,30
27	265,3	640	16,21	14,49	920	8538	15,83	402,6	11,18	1,22	0,087	1,13	10,05

28	414,7	962	9,47	30,48	1408	9946	8,99	219,9	11,73	2,57	0,059	2,51	9,21
29	320,0	614	0,41	28,68	963	8970	-	-	15,39	3,04	0,000	3,04	12,34
30	176,7	381	0,34	9,18	567	7500	-	-	-	-	-	-	-
31	368,6	1081	1,88	34,01	1484	11831	1,34	35,6	15,93	3,90	0,014	3,89	12,03
32	531,9	992	1,01	29,77	1555	11903	-	-	21,50	4,70	0,000	4,70	16,80
33	368,3	818	0,46	29,85	1216	11059	-	-	12,97	0,78	0,000	0,78	12,18
34	344,9	602	0,47	22,69	970	10237	-	-	-	-	-	-	-
35	427,3	1498	31,41	42,45	1968	12661	30,76	746,6	18,76	4,76	0,141	4,62	14,14
37	318,7	730	7,99	31,21	1080	10589	7,51	204,7	11,68	0,77	0,046	0,72	10,95
38	309,1	1009	26,49	16,19	1335	11845	25,94	626,4	12,29	0,74	0,132	0,61	11,68
39	476,7	1167	6,52	29,64	1674	11789	5,93	139,8	16,80	4,03	0,038	3,99	12,81
40	476,3	966	20,19	22,49	1466	12134	19,64	489,1	15,86	3,32	0,114	3,20	12,66
41	424,7	709	0,40	29,24	1163	10568	-	-	12,13	1,00	0,000	1,00	11,12
42	609,2	1156	26,06	53,43	1819	12264	25,46	594,3	17,24	4,67	0,148	4,52	12,72
43	286,3	593	0,47	27,99	908	9755	-	-	12,93	1,15	0,000	1,15	11,78
44	341,8	584	0,47	23,92	950	9160	-	-	12,18	0,18	0,000	0,18	12,01
45	499,9	1117	0,50	30,40	1648	12234	-	-	19,71	4,92	0,000	4,92	14,79
46	375,8	733	0,46	19,98	1130	9892	-	-	-	-	-	-	-
47	322,5	634	0,45	13,22	970	10539	-	-	-	-	-	-	-
48	474,9	738	2,80	40,23	1253	11210	2,25	56,4	22,33	3,69	0,030	3,66	18,67
49	326,2	720	5,10	15,88	1063	9942	4,64	120,0	12,03	-0,14	0,055	-0,19	12,22
50	335,6	625	2,31	17,46	978	9053	1,89	54,1	13,69	0,94	0,019	0,92	12,77
51	495,2	864	2,61	30,74	1391	11482	2,05	55,6	19,62	4,73	0,024	4,71	14,91

***Energy values were obtained in calorimeter.

Table 5. Fetus measurements. # = cow code, FL = maternal feeding level, DIP = days in pregnancy at slaughter, BW = fetus body weight (kg), EvBW = eviscerated body weight (kg), Head (g), Feet (g), Carcass (g), Viscera (g), Reproductive apparatus (g), BL = body length (cm), TC = thoracic circumference (cm), HS = height at shoulder (cm), HR = height at rump (cm), CCE = cranial circumference eyes (cm) and CCN = cranial circumference neck (cm).

#	FL	DIP	BW	EvBW	Head	Feet	Carcass	Viscera	Reproductive	BL	TC	HS	HR	CCE	CCN
1	High	240	17,84	14,06	1811	1401	10845	1660	64,40	77,1	54,2	67,2	66,3	43,9	44,5
3	High	189	6,66	5,07	876	336	3861	656	24,40	54,9	38,2	39,5	38,5	33,9	32,7
5	Low	139	1,61	1,20	250	66	884	196	7,91	32,5	24,5	24,0	22,0	23,4	23,0
6	Low	234	21,51	17,53	2168	1567	13792	2009	80,30	79,8	59,9	62,4	65,8	47,7	41,7
8	High	269	27,51	22,50	2636	2037	17831	2521	62,30	86,2	68,9	76,2	75,7	53,6	50,5
10	High	265	33,27	24,85	2628	2038	20184	3475	81,90	95,2	69,6	76,2	75,2	51,6	51,6
11	Low	138	1,78	1,30	252	72	980	184	8,17	34,5	24,0	23,5	22,5	23,0	21,6
12	Low	269	21,70	16,69	2020	1617	13052	2204	55,90	81,8	60,9	71,8	72,9	47,3	46,4
13	High	189	5,51	4,21	705	319	3181	557	21,30	50,5	37,0	36,9	36,5	31,5	30,1
14	Low	272	27,59	19,95	2420	2142	15392	2768	99,40	85,8	65,6	73,9	72,4	51,3	45,8
15	Low	135	1,47	1,12	243	54	825	168	7,49	32,5	24,3	24,0	22,8	22,5	22,4
16	High	191	7,70	6,01	820	416	4773	716	29,40	57,0	44,1	45,6	44,9	36,2	33,9
17	Low	188	5,30	3,84	819	282	2740	529	15,10	47,8	40,8	35,8	40,0	34,3	33,0
18	Low	266	25,67	20,31	2327	1716	16270	2435	103,60	80,8	61,6	72,8	76,4	46,3	46,3
19	High	236	20,10	15,75	1860	1543	12346	2098	61,70	80,0	58,5	63,4	65,6	49,0	42,6
20	High	140	2,02	1,56	314	94	1150	254	7,61	36,2	26,8	25,8	23,9	24,9	24,4
22	Low	240	21,94	18,31	1888	1829	14593	2039	59,00	79,7	61,5	69,1	67,6	46,6	43,5
24	Low	269	22,69	18,54	2101	1609	14825	2288	76,00	85,9	60,5	67,4	74,0	50,0	44,9
26	High	268	31,60	25,43	2515	2155	20755	2827	105,40	95,3	63,5	80,3	81,2	51,7	48,2
27	Low	241	13,99	11,26	1560	938	8760	1455	29,63	66,5	50,9	62,5	63,5	41,2	40,4
28	Low	190	8,09	6,20	983	464	4749	858	36,70	58,3	39,9	43,1	42,7	35,8	34,0
31	High	134	1,17	0,88	202	49	624	139	7,70	31,2	22,2	21,7	21,0	21,7	20,5
35	High	270	26,53	21,90	2372	1453	18075	2463	57,50	82,4	61,7	75,8	78,2	47,9	47,7
37	Low	192	7,11	5,38	866	430	4081	770	30,00	55,7	42,2	42,1	42,1	38,5	32,4
38	Low	239	22,69	17,56	1993	1755	13811	2035	76,80	80,8	60,6	69,3	66,2	47,3	43,3
39	High	191	6,00	4,52	756	337	3429	608	20,50	52,0	36,4	41,2	41,1	33,9	30,6

40	High	240	19,84	15,70	1880	1495	12320	1869	37,29	79,5	56,0	64,2	63,0	49,5	44,3
42	High	240	21,34	17,61	1970	1625	14010	1930	51,52	77,0	59,3	66,0	61,0	46,0	45,0
48	High	130	0,99	0,75	176	37	540	119	6,15	28,2	21,1	19,5	18,7	20,6	20,0
49	Low	185	4,25	3,26	597	203	2457	475	15,80	44,5	35,8	34,9	33,9	32,4	29,3
50	Low	134	1,34	1,01	230	55	726	166	8,71	30,5	23,5	22,5	20,5	21,5	22,5
51	High	135	1,64	1,23	268	60	906	165	7,15	34,0	25,8	25,2	24,2	25,0	23,2