ANNA LUIZA LACERDA SGUIZZATO

CHANGES IN MATERNAL BODY COMPOSITION, DEVELOPMENT OF THE CONCEPTUS AND ESTIMATION OF ENERGY REQUIREMENTS FOR PREGNANT CROSSBRED HOLSTEIN × GYR COWS

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

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APPROVED: July 23, 2018.

Alex Lopes da Silva

Fernanda Samarini Machado

Marcos Inácio Marcondes
(Co-adviser)

Polyana Pizzi Rotta
(Adviser)

In memory of my grandmas Lili and Nair, who always taught me to never lose the faith. They had a remarkable strength for living, even in the worst moments.

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BIOGRAPHY

Anna Luiza Lacerda Sguizzato daughter of Sonia Maria Lacerda Sguizzato and Mauro Sguizzato, was born in December 19, 1991 in São Paulo – SP, Brazil.

She started her undergrad in *Universidade Federal de Viçosa* in 2010 and became a Bachelor of Science in animal Science in 2016. During her undergrad, she had the privilege to participate of the program “Science without Boarders”. She spent a year and half study at University College Dublin in Dublin, Ireland.

In 2016, she started the M.S. program in *Universidade Federal de Viçosa*, with major on ruminant nutrition and dairy cattle production.

Today, July 23, 2018 she submits her dissertation to obtain the *Magister Scientiae* degree in Animal Science.

SUMMARY

ABSTRACT

SGUIZZATO, Anna Luiza Lacerda, M.Sc., Universidade Federal de Viçosa, July, 2018. **Changes in maternal body composition, development of the conceptus and estimation of energy requirements for pregnant crossbred Holstein × Gyr cows.** Advisor: Polyana Pizzi Rotta. Co-advisor: Marcos Inácio Marcondes.

This study aimed to evaluate the effects of plane of nutrition and advancing days of pregnancy (**DP**) on maternal body composition, fetal development and energy requirements of pregnant crossbred Holstein × Gyr cows. Differing planes of nutrition were established by two feeding regimes (**FR**): ad libitum (**AL**) or maintenance (**MA**). Sixty-two nonlactating multiparous Holstein × Gyr cows with average body weight (**BW**) of 480 ± 10.1 kg and an age of 5 ± 0.5 years were used. Cows were divided into three groups: pregnant (n = 44), non-pregnant animals (n = 12), and base line reference cows (n = 6). The 56 pregnant and non-pregnant cows were randomly allocated into two different FR: AL and MA. Cows fed at MA received 1.15% of their BW on a dry matter (**DM**) basis. Receiving corn silage and a concentrate-based diet at a ratio of 93:7 on a DM basis. Reference group cows were slaughtered at the beginning of the experimental period to estimate body composition and empty body weight (**EBW**). To evaluate the effects DP, pregnant and non-pregnant animals were slaughtered at day 140, 200, 240, and 270 of gestation. Feeding regimen affected maternal tissue (**MT**) composition and DP changed fresh weight (**FW**), DM and energy content, but no differences were observed for crude protein (**CP**) and ether extract (**EE**) in response to DP. Feeding regimen affected mammary gland components (CP, EE, and energy content), but not fresh or dry weights. Days of pregnancy influenced almost all mammary gland components, except energy content. In regard to the uterus, FR affected only fresh and dry weights; however, DP impacted every uterus component measured. The only interaction between FR and DP in this study was observed for placental fresh weight. Cows fed AD on d 270 presented the same placental FW as cows at MA and AD on d 200 and 240. Further, pregnant cows fed at MA on d 270 had greater placental FW than cows fed AD at this day. Days of pregnancy, but not FR, influenced composition of fetal fluids in pregnant cows. Finally, cows fed at MA had greater FW for fetus than cows fed AD; however, fetus composition changed over DP. It was observed an increase for FW, DM, EE and energy content until day 270, but a decrease in CP. Requirements for maintenance did differ between pregnant and non-pregnant cows, thus two equations were developed. Net energy (**NE**) and metabolizable energy (**ME**) requirements for maintenance of non-pregnant cows estimated in this study were 81 kcal/EBW$^{0.75}$/d and 131 kcal/EBW$^{0.75}$/d, respectively. The efficiency of use of ME for maintenance of non-pregnant cows was 61.8%. Net energy and ME for maintenance of pregnant cows were 85 kcal/EBW$^{0.75}$/d and 136 kcal/EBW$^{0.75}$/d, respectively. Efficiency of use of ME for maintenance of pregnant cows was 62.5%. Estimates of net energy for gain (**NE$_g$**) were different using non-pregnant and pregnancy cows' data, thus non-pregnant cows' requirements are recommended. The efficiency of use of ME for gain was 41.9%. Energy requirements for pregnancy were estimated fitting energy in gestation components (Mcal) in function of DP using a non-linear regression. The efficiency of use of ME for pregnancy was 15%. Furthermore, net energy for pregnancy was statistically significantly different from zero from day 70 of pregnancy. In conclusion, the novelty of our data presents specifically how changes due to feeding regime and days of pregnancy occur in maternal tissues and the conceptus. Net energy and ME for maintenance of non-pregnant Holstein × Gyr cows are different from pregnant cows. Regarding NE$_g$, we recommend estimating it only with data from non-pregnant animals. Furthermore, we believe that the proposed non-linear equations to estimate net energy requirements for pregnancy are more adequate and should be recommended for Holstein × Gyr cows.

RESUMO

SGUIZZATO, Anna Luiza Lacerda, M.Sc., Universidade Federal de Viçosa, julho de 2018. **Alterações na composição corporal materna, desenvolvimento do concepto e estimativa das exigências energéticas para vacas gestantes Holandês × Gir.** Orientador: Polyana Pizzi Rotta. Coorientador: Marcos Inácio Marcondes.

Objetivou-se avaliar os efeitos do plano nutricional e o avanço dos dias de gestação sobre a composição corporal materna, o desenvolvimento fetal e estimar as exigências de energia de vacas mestiças Holandês × Gir durante a gestação. Diferentes regimes alimentares foram estabelecidos de acordo com duas dietas: *ad libtum* (**AD**) e mantença (**MA**). Foram utilizadas 62 vacas multíparas da raça Holandês × Gir, não lactantes, com peso corporal médio (PV) de 480 ± 10,1 kg e idade de 5 ± 0,5 anos. Estes animais foram divididos em três grupos: animais gestantes (n = 44), animais não gestantes (n = 12) e animais referência (n = 6). As 56 vacas gestantes e não gestantes foram alocadas aleatoriamente em dois grupos diferentes: AL e MA. Os animais alimentados na MA receberam 1,15% do seu PC com base na matéria seca (**MS),** recebendo silagem de milho e dieta à base de concentrado na proporção de 93: 7 com base na MS. Os animais do grupo referência foram abatidos no início do período experimental para estimar a composição corporal e o peso de corpo vazio (**PCVZ**). Para avaliar os efeitos dos DG, animais gestantes e não gestantes foram abatidos nos dias 140, 200, 240 e 270 de gestação. O regime alimentar afetou a composição do tecido materno (**TM**) e os DG alteraram a matéria natural (**MN**), a MS e conteúdo energético, mas não foram observadas diferenças para proteína bruta (**PB**) e extrato etéreo (**EE**) em resposta aos DG. O regime alimentar afetou os componentes da glândula mamária (PC, EE e conteúdo energético), mas não MN ou MS. Dias de gestação influenciaram quase todos os componentes da glândula mamária, exceto o conteúdo energético. Em relação ao útero, o regime alimentar afetou apenas a MN e MS. Entretanto, os DG impactaram em cada componente do útero. A única interação observada neste estudo foi entre regime alimentar e DP para o componente MN da placenta. Vacas alimentadas AD no d 270 apresentaram a mesma MN que vacas alimentadas na MA e AD no d 200 e 240. Além disso, vacas prenhes alimentadas na MA no d 270 apresentaram maior MN placentária do que vacas alimentadas com AD neste mesmo período. Os dias de gestação, mas não o regime alimentar, influenciaram a composição dos líquidos fetais em vacas gestantes. Finalmente, as vacas alimentadas na MA apresentaram maior MN para o feto do que as vacas alimentadas AD. No entanto, houve mudança na composição corporal fetal em relação aos DG. Foi observado um aumento para o MN, MS, EE e conteúdo energético até o d 270, mas uma diminuição na PB. Houve diferença na exigência de mantença entre vacas gestantes e não gestantes, desta forma duas equações foram desenvolvidas. As exigências de energia líquida (**EL**) e energia metabolizável (**EM**) para mantença de vacas não gestantes estimadas neste estudo foram de 81 kcal/PCVZ^{0,75}/dia e 131 kcal/PCVZ^{0,75}/dia, respectivamente. A eficiência do uso de EM para mantença de vacas não gestantes foi de 61,8%. A energia líquida e a EM para mantença de vacas gestantes foi de 85 kcal/PCVZ^{0,75}/dia e 136 kcal/PCVZ^{0,75}/dia, respectivamente. A eficiência do uso de EM para mantença de vacas gestantes foi de 62,5%. As estimativas de energia líquida para ganho ($\mathbf{EL_g}$) foram diferentes para vacas gestantes e não gestantes, portanto, recomenda-se o uso da equação de vacas não gestantes. A eficiência do uso de EM para ganho foi de 41,9%. As exigências de energia para a gestação foram estimadas ajustando o conteúdo energético do componente gestação (Mcal) em função dos dias de gestação usando uma regressão não linear. A eficiência do uso de EM para gestação foi de 15%. Além disso, a energia líquida para gestação foi estatisticamente diferente de zero no dia 70 de gestação. Em conclusão, os dados obtidos neste trabalho apresentam, especificamente, como as mudanças devido ao regime alimentar e aos dias de gestação ocorrem nos tecidos maternos e no concepto. Ademais, observamos que há diferença, na exigência de energia líquida e energia metabolizável, para vacas gestantes e não gestantes Holandês × Gir. Em relação a EL$_g$, recomendamos utilizar a equação estimada com animais não gestantes. Acreditamos ainda que a equação não linear proposta para estimar a exigência de energia líquida para vacas gestantes é mais adequada e deve ser recomendada para vacas gestantes Holandês × Gir.

GENERAL INTRODUCTION

Changes during pregnancy are enormous for every single species. At this time, modifications on mother tissue, partitioning of nutrients and requirements for the dam and the conceptus is of utmost importance, nevertheless, they are difficult to account (Hammond, 1947; Gionbelli, 2013). There are few studies to estimate energy requirements during the gestational period of bovines, also to evaluate changes in mother tissue composition, development of fetus, mammary gland, uterus and fetal tissues due to gestation (Ferrell et al., 1976b; c; Bell, 1995; Bell et al., 1995), mainly for crossbred animals.

Crossbred animals can be widely found in South American, Asian and African continents. This crossing is a great strategy used in tropical climate countries, enabling higher rusticity and capability to adapt to harsh environments. For example, India holds approximately 82% of the herd in Asia (Trivedi and Steane, 2017) and their first option for crossing are the Holstein and Gyr breeds. In Africa, there were efforts to introduce more productive exotic breeds, partially to replace local cattle and partially for crossing with native ones (Mahadevan, 1965). These exotics breeds are now found in almost all the African territory (Khan et al., 2011)
According to Ruas et al. (2011), 74% of the Brazilian dairy herd is composed of crossbred Holstein × Zebu cows and a small number of animals in the national dairy herd is of specialized cows, mainly Holstein, the remaining animals are not categorized. Crossbred dairy animals are considered the result of breeding a Zebu breed with a pure European one, with the advantage of higher heterosis and complementarity when compared to the pure breeds (Jacopini et al., 2016; Ribeiro et al., 2017; Fialho et al., 2018). Therefore, to enhance these animals' production it is important to meet their nutrient requirements.

Animals have nutritional demands to keep their body working (maintenance requirements), to grow (growth requirements), to reproduce (pregnancy requirements), and to produce (lactation requirements). To meet these animals' demand for nutrient, some requirements systems were created INRA (1978), AFRC (1993), NRC (2001) and CSIRO (2007), French, English, American and Australian, nutrient requirements system for dairy cattle, respectively. Thus, professionals in the field of dairy cattle nutrition, utilize the available information from international systems to formulate diets on the absence of data for Holstein × Gyr animals.

Nevertheless, the BCNRM (2016) reported differences of energy requirements for maintenance between *Bos indicus* and *Bos taurus* breeds. Furthermore, Oliveira (2015) found unsatisfactory results trying to validate available systems in tropical conditions with crossbred dairy animals (European × Zebu). These middling results, as suggested by Oliveira (2015), could be due to lower metabolizable energy requirements for maintenance and reduced net energy efficiency for milk production when compared to pure *Bos taurus* breeds.

Net energy requirements for maintenance can be understood as the total heat production of a fasting animal, achieving their basic functions as the homeostasis and voluntary activities, without changing its corporal composition (Garret el al., 1959). However, some factor can affect energy requirements for maintenance, as breed, sexual class, quantity of activity, feeding system and temperature (BCNRM, 2016). According the same authors, bulls require higher energy for maintenance than steers and heifers. The AFRC (1993) suggests an increase of 10 and 20% of energy for beef and dairy cattle, which are not in feedlots.

There is also a vast literature related to the possible changes in organs size and visceral fat accumulation towards maintenance requirements (Solis et al., 1988;

Ferrell and Jenkins, 1998; Oliveira, 2015). It is suggested that an increase in organ size and greater amounts of visceral fat can enhance energy requirements, because of their greater metabolic rates. Mainly in dairy breeds, which is known to have more internal fat than beef breed. However, there is little information related to size of internal organs accumulation of visceral fat in pregnant animals. To our knowledge, there is a study from Ferrell et al. (1976c) affirming no changes in organs size due to pregnancy, but changes related to feeding regimen, increasing size of liver of heifer feed higher levels of energy. Despite these reduce information on maintenance requirements of pregnant animals, there is some indicatives of changes in body weight, liver and intestines sizes due to pregnancy (Silva, 2010).

Energy requirements for weight gain may be understood as the amount of energy retained in animals' body as protein or fat (Garrett et al., 1959). Thus, the composition of the gain is what will determine the requirements. Energy requirements for gain is also related to sexual class and breed. For example, according to the BR-CORTE (2016) Nellore steers have net energy requirements for gain (**NEg**) 14% greater than bulls and 7% smaller than females. Furthermore, for crossbred animals, NEg of steers is 12% greater than for bulls and 13% lower for females.

Nonetheless, energy requirements for maternal gain may be difficult to estimate during pregnancy because of the homeorhetic changes that the dam is undergoing (Bauman and Currie, 1980). Gionbelli et al. (2016) estimated energy requirements for maternal gain considering empty body gain of non-pregnant cows (**EBGnp**) and their body condition score (**BCS**). Consideration of these variables were thought to be more accurate than considering the whole body of the animal with fetus, conceptus and changes in mammary gland and uterus. Furthermore, his predictions were close to values obtained using BCNRM (2016) for *Bos taurus* breeds.

Although literature on nutrient requirements for maintenance, gain and milk production is reduced, requirements for pregnancy receive less attention than other subjects in requirements research. Energy requirements for pregnancy in all systems (INRA, 1978, AFRC, 1993, NRC, 2001 and CSIRO, 2007) were estimated a long time ago and animals underwent to an intensified selection for efficiency (Stephenson, 2017). In addition, the number of studies assessing energy requirements for pregnancy of Holstein × Gyr cows is even more reduced when compared to other data in literature (Lage, 2015). No requirement system consider a possible difference, or have any adjustment for this breed in question.

As an example, the NRC (2001) bases pregnancy requirements according to a study conducted by Bell et al. (1995). The study evaluated 18 multiparous cows, which were bred artificially using the same Holstein bull. The equation used to estimate this requirement takes into account days of gestation (**DG**) and the average weight of Holstein calves. However, the efficiency of metabolizable energy used by the uterus is assumed to be 0.14, the efficiency found by Ferrell et al. (1976c) in a study conducted with Hereford heifers. As a result, the energy requirement for pregnancy was considered 0 if DG were less than 190 and a maximum gestation length of 279 days, suggesting no increase in energy requirement after this period (NRC, 2001).

Regardless the impact of nutrient requirements during pregnancy, the estimation of these requirements is only possible with accurate values for rates of nutrient accretion in conceptus tissues and changes in maternal body (Bell et al., 1995). To have a greater knowledge of how nutrient accretion and changes in maternal body happens, it is important to understand nutrient partitioning. During pregnancy, the gravid uterus and the mammary gland has priority to receive nutrients when compared to other tissues in maternal body (Bauman and Currie, 1980).

The gravid uterus is assumed to be the sum of fetus, fetal fluids (amniotic and allantoic fluids), fetal membranes (blood vessels and umbilical cord), uterine tissues (myometrium and endometrium) and placentomes (cotyledons) (Bell et al., 1995). This nutrient priority from the gravid uterus and the mammary gland, during pregnancy, is called homeorhetic effect, which account for coordinated changes in metabolism of body tissue necessary to support a physiological state (Bauman and Currie, 1980), intrinsically related to pregnancy changes in animal's body. According to Ferrell et al. (1976b) and Bell (1995) the intensity of growth of the gravid uterus increase in the final third of gestation. Therefore, suggesting an increase in this homeorhetic effect due to pregnancy.

This intense partitioning of nutrients at the end of gestation associated with reduction in dry matter intake by the dam could result in mobilization of tissues to attend nutrient requirements of the gravid uterus. It is also suggested a reduction in size of intestines and rumen allowing space for fetal growth (Silva, 2010). However, Ferrell et al. (1976a) observed no differences in body composition of pregnant and non-pregnant heifers, only difference related to feeding regimen was observed.

To evaluate other changes in tissues due to pregnancy, some authors (Ferrell et al., 1976b; Bell et al., 1995; Gionbelli, 2013) considered a gestational component, which basically included the accretion of energy and protein in uterus due to pregnancy, the increase in mammary gland due to pregnancy and the accretion of placenta, fetal liquids, fetus and fetal membranes. In summary, the accretion of energy in all components, despite the fetus, is highly insignificant when it is transformed as energy requirements for fetus.

When accounting for energy requirements for gestation, we must consider all components and an important efficiency of conversion for metabolizable energy (**ME**) into net energy (**NE**) for gestation. In accordance with the data available, efficiency for gestation (**kgest**) is considerably low when compared to other efficiencies in metabolic processes (0.64 for maintenance and 0.60 for gain of non-lactating animals (NRC, 2001)). The kgest reported in the literature is 0.14 for Hereford heifers, Ferrell et al. (1976c); 0.12 for Nellore cows, Gionbelli (2013); 0.13 for Holstein × Gyr cows, Lage (2015). The explanation to the lower efficiency is related to the costs of maintaining conceptus products, because of their high oxidative rates (Lage, 2015).

Therefore, understanding the pattern of changes in maternal body, weight and deposition of tissues related to pregnancy is undoubtedly necessary to estimate requirements for pregnancy and provide an adequate nutrition for the dam and the fetus. Thus, the objective was to evaluate changes in body composition as weight gain or loss, rates of protein and energy deposition and estimated energy requirements of Holstein × Gyr cows during pregnancy.

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CHAPTER 1

Body composition changes of crossbred Holstein × Gyr cows and conceptus during pregnancy[1]

[1] Manuscript written to be submitted to the *Journal of Dairy Science*

ABSTRACT

This study aimed to evaluate the effects of plane of nutrition and advancing days of pregnancy (**DP**) on maternal body composition and fetal development. Differing planes of nutrition were established by two feeding regimes (**FR**): ad libitum or maintenance. Sixty-two nonlactating multiparous Holstein × Gyr cows with average body weight (**BW**) of 480 ± 10.1 kg and an age of 5 ± 0.5 years were used. Cows were divided into three groups: pregnant (n = 44), non-pregnant animals (n = 12), and base line reference cows (n = 6). The 56 pregnant and non-pregnant cows were randomly allocated into two different FR: ad libtum (**AL**) or maintenance (**MA**). Cows fed at MA received 1.15% of their BW on a dry matter (**DM**) basis. Receiving corn silage and a concentrate-based diet at a ratio of 93:7 on a DM basis. Reference group cows were slaughtered at the beginning of the experimental period to estimate body composition and empty body weight (**EBW**). To evaluate the effects DP, pregnant and non-pregnant animals were slaughtered at day 140, 200, 240, and 270 of gestation. Feeding regimen affected maternal tissue (**MT**) composition and DP changed fresh weight (**FW**), DM and energy content, but no differences were observed for crude protein (**CP**) and ether extract (**EE**) in response to DP. Feeding regimen affected mammary gland components (CP, EE, and energy content), but not fresh or dry weights. Days of pregnancy influenced almost all mammary gland components, except energy content. In regard to the uterus, FR affected only fresh and dry weights; however, DP impacted every uterus component measured. The only interaction between FR and DP in this study was observed for placental fresh weight. Cows fed AD on d 270 presented the same placental FW as cows at MA and AD on d 200 and 240. Further, pregnant cows fed at MA on d 270 had greater placental FW than cows fed AD at this day. In addition to placental changes, according to DP increased DM and energy content until day 270. Days of pregnancy, but not FR, influenced composition of fetal fluids in pregnant cows. Finally, cows fed at MA had greater FW for fetus than cows fed AD; however, fetus composition changed over DP. It was observed an increase for FW, DM, EE and energy content until day 270, but a decrease in CP. In conclusion, the novelty of our data presents specifically how changes due to feeding regime and days of pregnancy occur in maternal tissues and the conceptus.

Key words*:* gravid uterus, pregnancy, maternal tissue, nutrient deposition

INTRODUCTION

Pregnancy and lactation are high priority functions that have elevated nutrient demand (Bauman and Currie, 1980). During the final third of gestation, the developing fetus and mammary gland have increasing nutrient demands to support increased tissue growth and metabolic rate (Bauman and Currie, 1980; Bell, 1995). The change in metabolic rates for these tissues could bring enormous challenges to the cow, leading to early postpartum health problems and compromising lactation performance (Bell, 1995).

According to Bauman and Currie (1980), partitioning of nutrients to body tissues involves two types of regulation: homeostasis and homeorresis. Homeostasis is the capability to maintain a physiological equilibrium and/or constant conditions in the internal environment. The second type of regulation, homeorresis, accounts for coordinated changes in metabolism of body tissue necessary to support a physiological state (Bauman and Currie, 1980), which is intrinsically related to pregnancy changes in a body.

In order to know how changes occur during pregnancy, it is necessary to evaluate and estimate maternal body composition (Ferrell et al., 1976a). Therefore, the importance of obtaining data related to prenatal growth and tissue development during gestation is important to better understand the quality and quantity of nutrients required to sustain pregnancy (Ferrell et al., 1976b; Bell, 1995).

Nonetheless, there are limited studies evaluating body compositional changes in regard to pregnancy. To our knowledge, Ferrell et al. (1976a) and Bell (1995) were the only authors who studied the characteristics of these changes in the bovine mammary gland and gravid uterus. These authors (Ferrell et al., 1976a; Bell, 1995) were able to explain how nutrient absorption happens and how nutrient deposition takes place. However, to completely understand how the gestational process influences growth and requirements of pregnant cows, the following questions need to be addressed: What is the pattern of tissue deposition regarding fat and protein in pregnant cows? How do these components change over the lactation? Should differences in feeding regime be considered while evaluating nutrient requirements for pregnant cows? Does feeding regime affect maternal and fetal development? Research addressing these questions will provide a new perspective and allow a greater understanding of changes related to maternal and conceptus body composition during pregnancy.

Therefore, this research aimed to evaluate changes in maternal body composition and fetus development during different days of pregnancy (**DP**) when provided differing feeding regimes (**FR**): ad libitum or maintenance. We hypothesized that cows fed ad libtum will present greater fat deposition when compared to cows fed at maintenance, which will directly affect fetus development and tissue deposition from mid-gestation to peripartum.

MATERIAL AND METHODS

This study was conducted at Universidade Federal de Viçosa (Viçosa, MG, Brazil) following the standard procedures for humane animal care and handling according to the university's guidelines (47/2012).

Study data are from a previous experiment conducted by (Rotta et al., 2015a; b; c). Briefly, 62 Holstein × Gyr cows were used. Cows were divided into 3 groups: pregnant (n = 44), non-pregnant (n = 12), and baseline (n = 6). However, one abortion was verified in a cow from the ML treatment at 140 d. Thus, data from 43 cows were used for analyses, and 5 ML cows were evaluated at 140 d of gestation. Baseline cows were slaughtered at day 0. Pregnant and non-pregnant cows received two different feeding regimes (**FR**): ad libtum (**AD**) and maintenance [**MA**; 1.15% of body weight (**BW**)]. The 43 pregnant cows were slaughtered at four different days of pregnancy (**DP**): day 140, 200, 240, 270. However, 1 abortion was verified in a cow from the MA treatment at 140 d. Thus, data from 43 cows were used for analyses, and 5 ML cows were evaluated at 140 d of pregnancy. The 12 non-pregnant cows were slaughtered at day 200, 240, 270. Figure 1 provides a greater illustration of experimental scheme, FR and slaughtering.

For all comparisons between non-pregnant and pregnant animals, and among pregnancy groups, for the purpose of statistical tests, animals slaughter between 137 and 144 DP were included in the model as 140 days. Animals slaughtered between

196 and 201 days were included in the model as 200 days, animals slaughtered between 236 and 247 days were included in the model as 240 days. Lastly, animals slaughtered between 266 and 270 days of pregnancy were included in the model as 270 days.

Cows were slaughtered at Universidade Federal de Viçosa by captive bolt stunning followed by exsanguination. Right after exsanguination, the gravid uterus was immediately collected and weighted. The gravid uterus was sectioned in cervix height, then dissected into fetus, fetal membranes, uterus, fetal fluids. Weight of fetal fluids was obtained by difference between gravid uterus minus fetus, fetal membranes and uterus (Figure 2A). Fetus, fetal membranes, uterus, and mammary gland were individually ground and sampled. Then a homogenized sample was created with uterus and fetal membranes. Samples from gravid uterus and mammary gland were maintained at -80ºC until further analyses. The initial colostrum produced by cows at the end of gestation was not removed from the mammary gland. Thus, colostrum was accounted as part of mammary gland weight and composition obtained from animals slaughtered at day 268 of pregnancy.

Sampling after slaughtering extended to carcass and non-carcass components. Carcasses were divided into two halves, then the left half of carcass was weighed and chilled at 4ºC for 24h. After cooling, carcasses were weighed to evaluate the cold carcass weight and cold carcass dressing percentage. Further, to compose the non-carcass sample, the four stomachs and small and large intestines were washed after slaughter and added to the internal organs, head, tail, hooves, trimmings, hide, and blood (Figure 2B). All components were ground, homogenized and sampled. Calculation of maternal tissue (**MT**) sample was conducted by adding results from the carcass and non-carcass component samples.

Samples of carcass, non-carcass, mammary gland, uterus, placenta, fetal fluids, and fetus were analyzed for DM (AOAC, 2000; method 934.01), crude protein CP (AOAC, 2000; method 981.10), EE (AOAC, 2006; method 945.16), and energy content using the equation proposed by ARC (1980). Values obtained for body components were calculated as the sum of fresh weight (**FW**), DM, CP, EE, and energy content. Fresh weight content (**FW**; Table 1 and 2) was calculated as percentage of empty body weight (**EBW**). Dry matter content was calculated as percentage of FW. In addtion, CP, and EE were obtained as a percentage of DM and finally, energy content was calculated as the percentage of energy in EBW. Values of FW, DM, CP, and EE are presented in percentage (%) and values for energy are presented as Mcal per kg. More details about management and laboratorial analyzes are available in (Rotta et al., 2015a; b; c).

Statistical Analyses

Data on maternal tissue, mammary gland, uterus, and components of gravid uterus composition were evaluated as a completely randomized design with a factorial arrangement 2 × 5, two FR (MA and AD) and five DP (0, 140, 200, 240 and 270), using PROC GLIMMIX of SAS (version 9.4; SAS Institute, Inc. Cary, NC). All variables had normal distribution according to Shapiro–Wilk's test. Degrees of freedom were corrected using the Kenward Rodger approximation. When an effect of DP was observed, the student "t" test was used to compare least square means. In the absence of interactions, the main effects of FR and DP are discussed. For all analyses, significance was declared when $P < 0.05$.

RESULTS AND DISCUSSION

Carcass and Non-Carcass Composition

No interactions between FR and DP were observed ($P > 0.05$; Table 1) for carcass and non-carcass components of FW, DM, CP, EE and energy content. Feeding regime did not affect ($P = 0.146$) FW of carcass; however, it affected DM, CP, EE and energy content. Animals fed AD present greater ($P = 0.001$) DM content as a percentage of FW than MA animals. On the other hand, MA cows had greater ($P = 0.001$) content of CP as a percentage of DM. In addition, MA cows also presented lower ($P = 0.001$) percentage of EE when compared to cows fed AD, followed by a lower ($P = 0.001$) content of energy as a percentage of EBW.

Regarding DP, it affected FW and DM content ($P = 0.001$ and $P = 0.002$, respectively; Table 1). Nevertheless, DP did not affect ($P \geq 0.182$) CP, EE, and energy content. Fresh weight decreased as DP increased ($P = 0.001$), without differences ($P = 0.123$) for d 0 and 140. These values reduced 2% on d 200, and further reduced by an additional 2 and 3% on d 240 and 270, respectively (Table 1). During pregnancy, cows mobilized almost 9% of their FW content as a percentage of EBW. For DM content, the pattern of change is different, where a reduction ($P = 0.002$) was observed from d 0 to 140 (4%), followed by an increase ($P = 0.008$) on d 200 until the end of pregnancy.

Feeding regime affected all non-carcass components ($P = 0.001$; Table 1). Cows fed AD had greater contents of FW, DM, EE, and energy content. However, CP percentage was greater for cows fed at MA; this pattern of deposition was also observed for CP among carcass components. Days of pregnancy affected FW ($P = 0.001$) and DM ($P = 0.013$), similar to carcass components. Fresh weight of non-carcass decreased ($P = 0.042$) on d 140; however, the percentage of FW remained the same (P = 0.058; Table 1) through d 140, 200, 240. Furthermore, there was another decrease (P < 0.001) on d 270. For DM, its content reduced (P = 0.020) on d 140 following an increase on d 200 (P = 0.008; Table 1), this increase remained unaltered until the end of pregnancy. (Table 1). In addition, non-carcass DM content followed the same pattern as the observed in carcass DM content.

These patterns of deposition in carcass and non-carcass components reflect the FR offered to these pregnant cows, similar to growing and fattening animals (Owens et al., 1995; Nürnberg et al., 1998). Thus, the AD regime allowed a difference in EE deposition for these animals, greater EE deposited into the carcass and more EE stored in the organs and viscera in the non-carcass. Changes in FW due to DP may be related to differences in water content, since there was no difference in CP, EE, and energy content. Additionally, it is important to note that the differences in DM content due to DP did not affect the CP and EE measurements.

Maternal Tissue Composition

No interactions between FR and DP were observed for CT composition (P > 0.20; Table 2). Feeding regime affected (P = 0.009) FW, DM, CP, EE and energy content. Cows feed AD had greater FW, DM, EE, and energy content, when compared to MA animals; however, CP content was greater for MA animals.

Days of pregnancy affected FW (P = 0.001), DM (P = 0.001) and energy content (P = 0.025). However, there was no difference for CP (P = 0.349) and EE (P = 0.391). The reduction on percentage of FW for maternal tissues due DP can be greater understood observing Figure 3. Mobilization on FW is observed through pregnancy, becoming more intense during the final third (Table 2; Figure 3). Dry matter percentage decreased ($P \leq 0.001$) on d 0 to 140; however, it was observed increasing deposition of DM ($P \leq 0.001$) from d 200 until the end of pregnancy (Table 2). Changes in energy content are market by accretion and mobilization ($P = 0.025$) through pregnancy (Table 2; Figure 3). Crude protein and EE content were not different among DP ($P \geq 0.349$; Table 2).

The lower proportion of CP in MT of AD animals clearly indicates greater amounts EE storage as a percentage of DM. According to Marcondes et al. (2016), animals achieve mature BW when their content of EE is about 25% of EBW. Furthermore, according to Owens et al. (1993), animals that have not achieved their mature weight, may deposit less EE as a proportion of weight gain. In our study, animals fed AD had an average of 25% of EE in EBW, for MA cows EE was closer to 19%. Therefore, our results indicate that cows fed AD had lower rates of protein deposition than MA cows because of greater ability to deposit EE in the carcass and non-carcass components due to a weight that was closer to maturity (Marcondes et al., 2016).

Ferrell et al., (1976a) observed that physiological state (pregnant and non-pregnant) did not affect composition of offal, carcass or EBW when evaluating body composition of pregnant Hereford heifers. However, the pattern of body tissue deposition presented by the previous authors does not agree with the pattern of our study, which presented differences on FW of non-pregnant and pregnant cows.

Since MT is the sum of carcass and non-carcass components, as expected, MT changes in FW and DM over DP followed the same pattern. Despite the lower magnitude of values regarding CP and EE in MT, which lead to the absence of statistical differences ($P \geq 0.349$), there was a numerical difference in percentages of these components due to DP. Nevertheless, when combining CP and EE to calculate energy content as a percentage of EBW, these numerical differences became significant (*P* = 0.025). Then, a slightly, but greater value of energy content on MT of non-pregnant cows was observed (Table 2).

In fact, changes in energy content of MT are minimal over the DP. Thus, we considered it difficult to obtain greater values of energy contents due to the lower number of animals allocated in each treatment. Therefore, using five/six replications per treatment (FR x DP) was a limitation due to ethics committee laws and a greater number of pregnant cows would restrict the conduction of this study.

Mammary Gland Composition

There were no interactions between FR x DP ($P \geq 0.408$) for mammary gland composition. Feeding regime affected composition of CP (*P* = 0.006) in mammary gland, as well as EE (*P* = 0.007) and energy content (*P* = 0.031; Table 2). Days of pregnancy affected (*P* = 0.001) FW, DM, CP and EE content in mammary gland, but no difference was observed for energy content (*P* = 0.117).

We can explain the greater value obtained for CP in MA when compared to AD by the same idea expressed for MT. The greater proportion of CP is a response for reduced levels of EE content in the mammary gland of MA cows, which also leads to lower energy content in mammary gland of MA regime.

The effect (*P* = 0.001) of DP on FW observed in our study probably occurred as a response to colostrum production and accumulation. According to Castro et al. (2011), colostrum production in ruminants begins several weeks before parturition, even prior to lactogenesis, with a considerable increase in IgG content in milk secretor cells, 1 to 3 d before parturition. This pattern of increase in percentage of FW is shown in Figure 4, where changes in mammary gland composition during d of pregnancy are presented.

Crude protein accretion in the mammary gland due to DP is greater than other components, mainly from d 240 to 270 ($P \leq 0.001$; Table 2). Despite the unclear pattern of tissue deposition during pregnancy, this CP accretion could be considered as a great indicative of the mammogenesis process, which occurs with ductal branching and alveoli development (Plath et al., 1997; Plath-Gabler, 2001; Akers, 2017). On the other hand, there is an abrupt decrease in EE content in the mammary gland. It is important to highlight that the average EE on d 0 is greater ($P = 0.007$) than on d 140, 200, and 240 ($P \leq 0.02$). Thus, before pregnancy, cows present higher levels of EE in the mammary gland, which is mobilized during the first trimester of pregnancy. After this period, from d 240 to 270, there was another decrease ($P < 0.001$) in EE content, approximately 40% lower than the initial value obtained for EE at d 0.

In a previous study evaluating udder composition of pregnant and non-pregnant heifers, Ferrell et al. (1976b) found an increase in FW, DM, EE, and energy content (kg) at greater feeding levels. They observed an interaction between pregnancy and time in the case; in addition, they observed difference for udder FW. However, they did not observe changes in DM, EE, and energy content related to pregnancy, indicating little effect of mammary gland in requirements for pregnancy.

As suggested by Ferrell et al. (1976b) findings, our data showed changes in mammary gland composition due to feeding regime, with increased rates of EE for AD fed cows. What would allow us to question whether this long-term AD regime would impair future lactation, since AD regime affects the whole composition of mammary gland? In addition, the novelty of our data suggests a new point of view in the field of "changes in body composition of pregnant cows and conceptus development". However, our data points to increased FW, DM, CP, and EE due to

DP, indicating one more factor that requires nutrients for development during pregnancy.

Uterus Composition

No interactions for uterine composition measurements were observed for FR and DP ($P \geq 0.101$; Table 2). Feeding regime affected FW and DM in uterus composition ($P = 0.001$ and $P = 0.001$, respectively). Regarding DP, differences ($P = 0.001$) were observed for all components measured, FW, DM, CP, EE, and energy content (Table 2).

The uterus FW content for cows fed at MA was greater than cows fed AD. However, DM content resulted in a different pattern, with greater values for DM obtained in cows fed AD. This greater value obtained for FW could be due to compensatory stimuli from MA cows. Additional information about these characteristics were published by Rotta et al. (2015b), where they also found an increased number of placentomes in MA cows.

Uterine FW becomes greater as DP increase, achieving a percentage of 1.85 of cow's EBW at d 270. This increase is expected due to the natural development of fetus, placenta, and increased fetal fluids (Figure 5). On the other hand, uterus DM does not change according to pregnancy. Indeed, DM content on d 0 is greater ($P < 0.001$) than the measurements during pregnancy.

The pattern of CP deposition of in the uterus may be observed in Figure 5. Crude protein increases ($P > 0.001$) from d 0 to 140 followed by a slight and non-significant reduction until d 270. According to Bell (1995), there is an elevated uterine uptake of amino acids during gestation to supply pregnancy requirement; however, in the present study, this uptake was only observed until d 140, with a considerable retention of this nutrient until the end of pregnancy ($P \leq 0.963$). Ferrell et al. (1976b) also observed a similar pattern with heifers, which they attributed to a uterine growth that preceded growth of fetal membranes, supporting the fact that fetal development is dependent upon prior development of the uterus and fetal membranes (Ferrell et al., 1976b).

Ether extract content in the uterus was greater ($P \leq 0.004$) for cows on d 0 than as pregnancy increased. As a supposition, the greater fat content in uterus of non-pregnant cows could be used as an energy source to help uterus development, since uterine fat content is reduced by d 140 of gestation. This period of EE utilization as a gravid uterine energy source, coincides with an increase in gravid uterine CP deposition, which suggests greater amino acid uptake by this organ during early pregnancy.

Placenta Composition

An interaction between FR and DP was observed for FW component ($P = 0.001$). Feeding regime did not affect ($P \geq 0.267$) DM, CP, or EE, but did affect FW ($P = 0.001$) and energy content ($P = 0.025$). On the other hand, differences due to DP were observed for FW ($P = 0.001$), DM ($P = 0.001$), and energy content ($P = 0.001$). Placenta content of CP and EE did not differ according to DP ($P \geq 0.141$; Table 2).

As presented in the results, cows fed at MA presented a greater development of the placenta when compared to cows fed AD due to the interaction between FR and DP. Evaluating interaction results, we observed that cows fed AD or at maintenance on d 140 presented no differences ($P = 0.804$) on placenta FW; however, it was lower at d 140 than on d 200, 240, and 270. Then, from d 200 to 240, cows fed at MA and AD had no differences ($P \geq 0.06$) on placenta FW. In addition, cows fed AD on d 270 presented the same ($P \geq 0.183$) placental FW as cows at MA and AD on d 200 and 240. Further, pregnant cows fed at MA on d 270 had greater ($P < 0.001$) placental FW than cows fed AD at this day. Figure 6 provides a clear illustration of this interaction.

The placenta is formed by the connection between maternal and fetal tissues (Peter, 2013; Haeger et al., 2016). Nutrient exchange between the dam and fetus occurs through the placenta (as uptake of glucose and amino acids by the fetus; Bell et al., 1995). The placenta also works as a barrier against microbes and maternal immunological attack (Peter, 2013). Furthermore, in studies with ewes, the placenta is the most important tissue affecting fetal growth, mainly due to intrauterine growth restriction (Carr et al., 2016).

The increase in placental FW due to FR and DP may be due to greater number of placentomes observed for MA animals, and because of greater placental vascularization. A reported by Rotta et al. (2015b), cows fed at MA may have an adaption mechanism to supply requirements to the fetus through the placenta, without impairing fetal development.

The great increase in DM deposition from d 0 to 140 (Figure 7), corroborates the idea of the importance of placental development during early pregnancy. As pointed by Greseth et al., (2016), in early-gestation fetus survival relies upon nutrient transporters in the utero and the developing placenta, such as fructose and amino acids transporters. This dependency for nutrient transporters occurs because of the absence of umbilical blood flow, which is not developed in early pregnancy (Greseth et al., 2017). Therefore, the increase in placental DM on d 140, observed in our study, could be a response to increased requirement for nutrients.

Although the need for nutrients raises through pregnancy, the absence of differences ($P \geq 0.141$) regarding CP and EE in the placenta was noticed. However, we may not infer that there was no nutrient deposition in the placenta, once its FW, DM and energy content increased ($P = 0.001$) during DP. What we could infer, is that nutrients are deposited and utilized in different forms, as not only CP and EE. Therefore, a well-developed placenta with greater nutrient transporters, vascularity, and greater number of placentomes would be able to allow an appropriate fetal development (Rotta et al., 2015b).

Amniotic Fluid Composition

No interactions between FR and DP were observed in this study ($P \geq 0.301$). In addition, FR did not influence composition of fetal fluids in pregnant cows ($P \geq 0.140$). Nevertheless, DP affected FW of fetal fluids ($P = 0.026$), CP ($P = 0.031$), and energy content ($P = 0.035$). Differences were not observed regarding DP on DM ($P = 0.329$) and EE ($P = 0.686$; Table 2).

Fetal fluids are substantial for fetal development, due to fetal fluids physiological and nutritive response (Silva, 2010). These physiological and nutritive responses occur because of amniotic fluid composition, which include hormones, nutrients, and various solutes that may pass the placenta (Peter, 2013). According to Peter (2013), the volume of these fluids is related to DP and fetal membranes. In our study, the percentage of FW, CP and energy content in fetal fluids was significantly affected by DP ($P \leq 0.035$), which converge with the affirmatives of the two authors mentioned above.

There is a substantial increase of fetal fluid content at the end of pregnancy, approximately 2.5 times the initial average (Table 2), which is illustrated in Figure 8. Crude protein content increased ($P = 0.009$) until d 200, remaining the same until d

270. This increase in CP content converges with fetal development through pregnancy, indicating the need for nutrients as amino acids and fructose, which are known as primarily sources of energy utilized by the fetus (Bell et al., 1992, 1993; Greseth et al., 2016)).

A more recent research (Greseth et al., 2016), reported an increase of fructose on amniotic fluids due to maternal nutritional status and day of gestation (until d 50). They observed greater amounts of fructose at d 34 of cows fed a restricted diet, with considerable decrease at d 50. These authors considered these changes on amniotic fluids as a response to an increased demand previous to organogenesis, followed by a reduction. However, in our study, the period where an increase on fetal fluids occurs is after the organogenesis process (d 140 to 200). Therefore, greater changes happened in our study until d 200, where fetal fluids are more responsible to nourish the fetus. After this period, nutrition of fetus is more related to placenta, which explain the greater stability in percentage of fetal fluids.

Although we were not able to distinguish between the amniotic and allantoic fluids, Toniollo and Vicente (1995) observed lower amounts of amniotic fluids during the middle third of gestation and greater volume of allantoic fluids in the initial and final thirds of gestation. Data related to the composition of fluids collected by Toniollo and Vicente (1995) is not available. However, according to data obtained in the current study, the increase of CP as a percentage of total fetal fluids is greater than EE; therefore, showing no pattern of accretion of fat. Our results are further sustained by the fact that permeability of fatty acids through the placenta is minimal (Bell et al., 1992).

Fetus Composition

No interactions were observed between FR and DP (*P* ≥ 0.114; Table 2). Feeding regime only affected FW of fetus (*P* = 0.039), without major changes in other components as published by Rotta et al. (2015b). Furthermore, DP affected FW (*P* = 0.001), DM (*P* = 0.001), CP (*P* = 0.019), EE (*P* = 0.002), and energy content (*P* = 0.001; Table 2).

Fetal FW of cows fed at MA was greater than for cows fed AD. This considerable difference in fetal fresh weigh could be due to adaptations on placenta development of cows fed at MA. According to results obtained from the current study, fetal FW as a percentage of cow EBW increased considerably. Fetal FW achieved an average of 6.5% of dam's EBW at d 270 of gestation (Table 2). Further, increased DM content through pregnancy and the pattern of deposition may be observed in Figure 8. The increase observed in energy content is associated with the increase in CP and EE content. Moreover, CP content decreased as pregnancy advanced, where at d 140, CP accounted for 74% of the DM content and decreased to 65% of DM content at d 270. These results do not mean a reduction on CP content, but a possible increase in other components since DM content was still rising.

Regarding conceptus metabolism, its development is greater explained when considering all of conceptus components, the gravid uterus (Bell, 1995). Further, for fetal development, it is essentially to consider changes in fetal composition, which occur by nutrient requirements and availability. According to Bell (1995), only 32% of the amino acid N taken-up by the late-gestation fetus is deposited as protein. Therefore, fetal requirement for metabolizable amino acids is three times the net requirement for fetal growth. Considering data available from our study, in order to deposit 65% of CP in fetal tissues, the fetus would require three times more protein from the dam. Therefore, increased amount of CP would be required to be addressed to changes in fetal composition through pregnancy.

The pattern of EE deposition in the fetus is available in Table 2 and Figure 9. An increase of EE concentration was observed from d 140 to 200 ($P = 0.016$). The amount of EE at d 140 represents 7.5% of DM content and approximately 13% at d 270. According to Bell, (1995), the nutritional requirement for fat deposition in the fetus is relatively inconsiderable, approximately 5% of fetal energy requirements. Moreover, there is a reduced deposition of fat in the fetus due to a reduced placenta permeability to fatty acids (short- and long-chain) and ketones (Bell et al., 1993). Therefore, despite the absence of data on composition of Holstein × Gyr fetus, the lower percentage of fetal EE observed in our study agrees with information found on literature.

CONCLUSION

In conclusion, cows fed ad libtum presented greater percentage of fat in maternal tissues than cows fed at maintenance. These changes in fat content were not dependent on days of pregnancy, restricting these modifications on maternal tissues to feeding regime solely. In addition to changes due to the feeding regimes offered, there were changes in the mammary gland, where cows fed ad libtum had greater percentage of fat than protein. However, changes in mammary gland composition occurred due to days of pregnancy, allowing us to imply the dependence upon the energy content in ether extract to the development of the mammary gland through pregnancy. Regarding uterus, placenta and fetal content, animals fed at maintenance presented greater percentages of these contents than animals fed ad libtum. Although there was a lower impact of feeding regime on the conceptus, significant changes were observed for days of pregnancy. The novelty of our data presents specifically how changes in maternal tissues occur, as much as changes on the conceptus. Therefore, new researches should be focused on pattern of accretion and mobilization of fetal fluids through pregnancy, how fat and protein change during lactation and what level of feeding will impair development of these tissues related to the conceptus.

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Table 1. Composition of carcass[1] and non-carcass[2] tissue according to feeding regime (FR) and days of pregnancy (DP).

Items[3]	Feeding Regime		Days of Pregnancy					SEM	*P*-value		
	Maintenance	*Ad Libtum*	0	140	200	240	270		FR	DP	FR×DP
	n = 29	n = 26	n = 12	n = 10	n = 11	n = 11	n = 11				
					Carcass						
FW (%)	58.670	57.946	61.997^{a}	60.759^{a}	58.712^{b}	56.771^{c}	53.300^{d}	0.771	0.146	0.001	0.221
DM (%)	40.994	43.594	43.047^{a}	39.021^{b}	42.467^{a}	42.518^{a}	44.418^{a}	1.182	0.001	0.002	0.427
CP(%)	39.650	34.083	37.547	38.751	35.721	36.589	35.724	2.307	0.001	0.676	0.183
EE(%)	53.496	63.348	56.574	56.337	60.532	58.574	59.685	3.371	0.001	0.655	0.149
Energy (Mcal/kg)	1.748	1.991	1.985	1.771	1.931	1.837	1.821	0.097	0.001	0.182	0.136
					Non-Carcass						
FW (%)	32.563	34.400	35.291^{a}	34.112^{b}	34.030^{b}	32.968^{b}	31.006^{c}	0.552	0.001	0.001	0.633
DM (%)	36.813	42.347	39.603^{a}	36.357^{b}	40.159^{a}	40.933^{a}	40.846^{a}	1.316	0.001	0.013	0.736
CP(%)	47.050	37.117	41.934	46.207	38.116	42.831	46.207	3.480	0.001	0.288	0.648
EE(%)	46.507	57.658	52.151	48.031	57.300	51.507	51.424	4.184	0.001	0.322	0.799
Energy (Mcal/kg)	0.845	1.097	1.024	0.894	1.034	0.985	0.917	0.057	0.001	0.067	0.781

[1] Carcass = bones and meat cuts from half carcass.

[2] Non-carcass = organs, stomachs, intestines, head, tail, hooves, trimmings, hide and blood.

[3] FW = fresh weight, DM = dry matter content, CP = crude protein, and EE = ether extract.

[a – d] Means associated with days of pregnancy within a row with different superscripts differ ($P \leq 0.05$).

Table 2. Composition of maternal tissue[1], mammary gland, uterus, placenta, fetal fluids, and fetus according to feeding regime (FR) and days of pregnancy (DP).

	Feeding Regime		Days of Pregnancy					SEM	P-value		
Items[2]	Maintenance	*Ad Libtum*	0	140	200	240	270		FR	DP	FR x DP
	n = 29	n = 26	n = 12	n = 10	n = 11	n = 11	n = 11				
Maternal Tissue											
FW (%)	91.232	92.346	97.288^{a}	94.871^{b}	92.743^{c}	89.739^{d}	84.305^{e}	0.638	0.009	0.001	0.247
DM (%)	39.513	43.157	41.822^{a}	38.118^{b}	41.644^{a}	41.941^{a}	43.139^{a}	0.953	0.001	0.001	0.437
CP (%)	42.025	35.031	38.900	41.073	36.480	36.680	37.508	2.182	0.001	0.349	0.454
EE (%)	51.255	61.433	55.169	53.591	59.509	56.444	57.004	2.965	0.001	0.391	0.249
Energy (Mcal/kg)	2.592	3.087	3.009^{a}	2.665^{c}	2.966^{ab}	2.822^{abc}	2.739^{bc}	0.116	0.001	0.025	0.208
Mammary Gland											
FW (%)	1.714	1.906	1.148^{c}	1.468^{bc}	1.598^{bc}	1.804^{b}	3.033^{a}	0.289	0.302	0.001	0.900
DM (%)	35.393	37.945	43.269^{a}	34.673^{b}	39.164^{ab}	38.785^{ab}	27.454^{c}	3.182	0.212	0.001	0.733
CP (%)	39.828	32.100	23.482^{c}	35.791^{b}	33.420^{b}	34.959^{b}	52.168^{a}	4.255	0.006	0.001	0.410
EE (%)	58.253	66.310	75.407^{a}	62.467^{b}	64.996^{b}	63.443^{b}	45.094^{c}	4.453	0.007	0.001	0.408
Energy (Mcal/kg)	0.044	0.055	0.042	0.040	0.050	0.055	0.059	0.008	0.031	0.117	0.782
Uterus											
FW (%)	1.128	0.942	0.147^{e}	0.525^{d}	1.061^{c}	1.600^{b}	1.844^{a}	0.082	0.001	0.001	0.101
DM (%)	14.099	15.218	18.605^{a}	14.221^{b}	13.633^{b}	13.697^{b}	13.134^{b}	0.545	0.002	0.001	0.588
CP (%)	84.220	82.779	80.327^{b}	86.126^{a}	83.481^{ab}	84.006^{a}	83.558^{ab}	1.665	0.179	0.025	0.963
EE (%)	9.985	11.695	15.674^{a}	8.014^{b}	10.516^{b}	9.970^{b}	10.025^{b}	1.745	0.129	0.001	0.981
Energy (Mcal/kg)	0.008	0.008	0.002^{d}	0.004^{c}	0.008^{b}	0.012^{a}	0.014^{a}	0.001	0.324	0.001	0.858
Placenta											
FW (%)	0.500	0.345	-	0.014^{c}	0.396^{b}	0.453^{b}	0.699^{a}	0.353	0.001	0.001	0.001
DM (%)	8.224	8.588	-	6.423^{c}	8.177^{b}	9.153^{ab}	9.871^{a}	0.777	0.513	0.001	0.067
CP (%)	81.858	86.388	-	75.338	86.372	87.775	87.007	5.659	0.267	0.141	0.376

EE (%)	4.136	4.151	-	4.082	4.169	3.736	4.586	0.528	0.969	0.441	0.540
Energy (Mcal/kg)	0.002	0.002	-	0.001^{c}	0.002^{b}	0.002^{b}	0.003^{a}	0.001	0.025	0.001	0.296
					Fetal Fluids						
FW (%)	2.616	1.631	-	1.331^{b}	1.633^{b}	1.609^{b}	3.922^{a}	0.921	0.140	0.026	0.520
DM (%)	1.181	1.341	-	1.240	1.445	1.173	1.186	0.168	0.188	0.329	0.873
CP (%)	26.080	28.554	-	18.127^{b}	32.902^{a}	25.983^{ab}	32.255^{a}	5.140	0.502	0.031	0.655
EE (%)	12.467	13.106	-	12.752	12.895	13.618	11.881	1.465	0.542	0.686	0.669
Energy (Mcal/kg)	0.001	0.001	-	0.0004^{b}	0.0007^{ab}	0.0005^{b}	0.0013^{a}	0.000	0.421	0.035	0.301
					Fetus						
FW (%)	3.625	3.149	-	0.399^{d}	2.196^{c}	4.414^{b}	6.539^{a}	0.313	0.039	0.001	0.221
DM (%)	15.351	16.371	-	10.476^{d}	14.657^{c}	17.762^{b}	20.349^{a}	1.191	0.283	0.001	0.197
CP (%)	69.675	69.338	-	74.150^{a}	70.892^{ab}	67.917^{bc}	65.066^{c}	2.768	0.865	0.019	0.565
EE (%)	11.021	11.787	-	7.529^{b}	11.409^{a}	13.565^{a}	13.113^{a}	1.486	0.472	0.002	0.114
Energy (Mcal/kg)	0.033	0.029	-	0.002^{d}	0.016^{c}	0.041^{b}	0.064^{a}	0.003	0.119	0.001	0.598

[1] Maternal Tissue = sum of carcass and non-carcass components.

[2] FW = fresh weight, DM = dry matter content, CP = crude protein and EE = ether extract.

$^{a-e}$ Means within a row with different superscripts differ ($P \leq 0.05$).

FIGURES

Figure 1. Experimental scheme, feeding regime, and slaughter groups.

Figure 2. Components of gravid uterus and mammary gland (A) and cows' sub-divisions according to sampling at slaughter (B).

Figure 3. Changes in maternal tissues composition (MT) from d 0 through pregnancy. Accretion or mobilization in % of empty body weight, % of DM or Mcal per kg.

Figure 4. Changes in mammary gland composition from d 0 through pregnancy. Accretion or mobilization in % of empty body weight, % of DM or Mcal per kg.

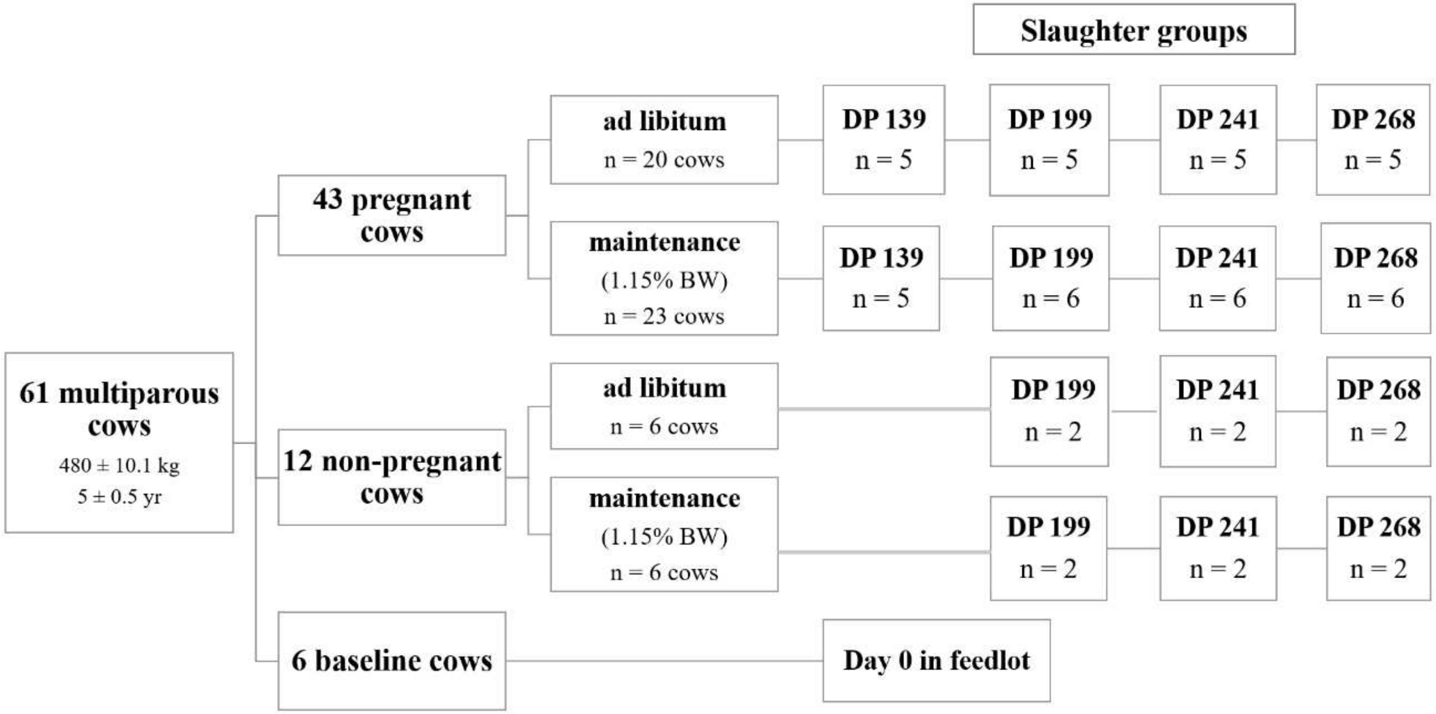
Figure 5. Changes in uterus composition from d 0 through pregnancy. Accretion or mobilization in % of empty body weight, % of DM or Mcal per kg.

Figure 6. Interaction between feeding regimen (FR) and days of pregnancy (DP) on placental fresh weight (% EBW) of cows fed at maintenance (MA) and ad libtum (AD).

Figure 7. Changes in placenta composition from d 0 through pregnancy. Accretion or mobilization in % of empty body weight, % of DM or Mcal per kg.

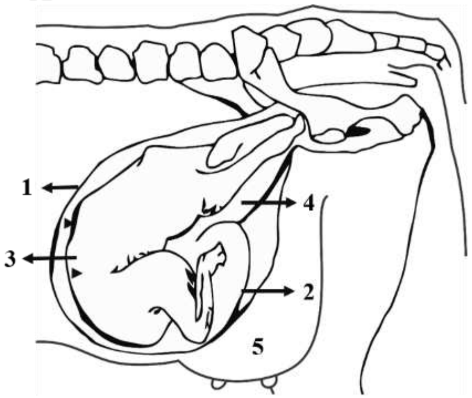
Figure 8. Changes in fetal fluids composition from d 0 through pregnancy. Accretion or mobilization in % of empty body weight, % of DM or Mcal per kg.

Figure 9. Changes in fetus composition from d 0 through pregnancy. Accretion or mobilization in % of empty body weight, % of DM or Mcal per kg.



Sguizzato, figure 3. Experimental scheme, feeding regime, and slaughter groups.

A



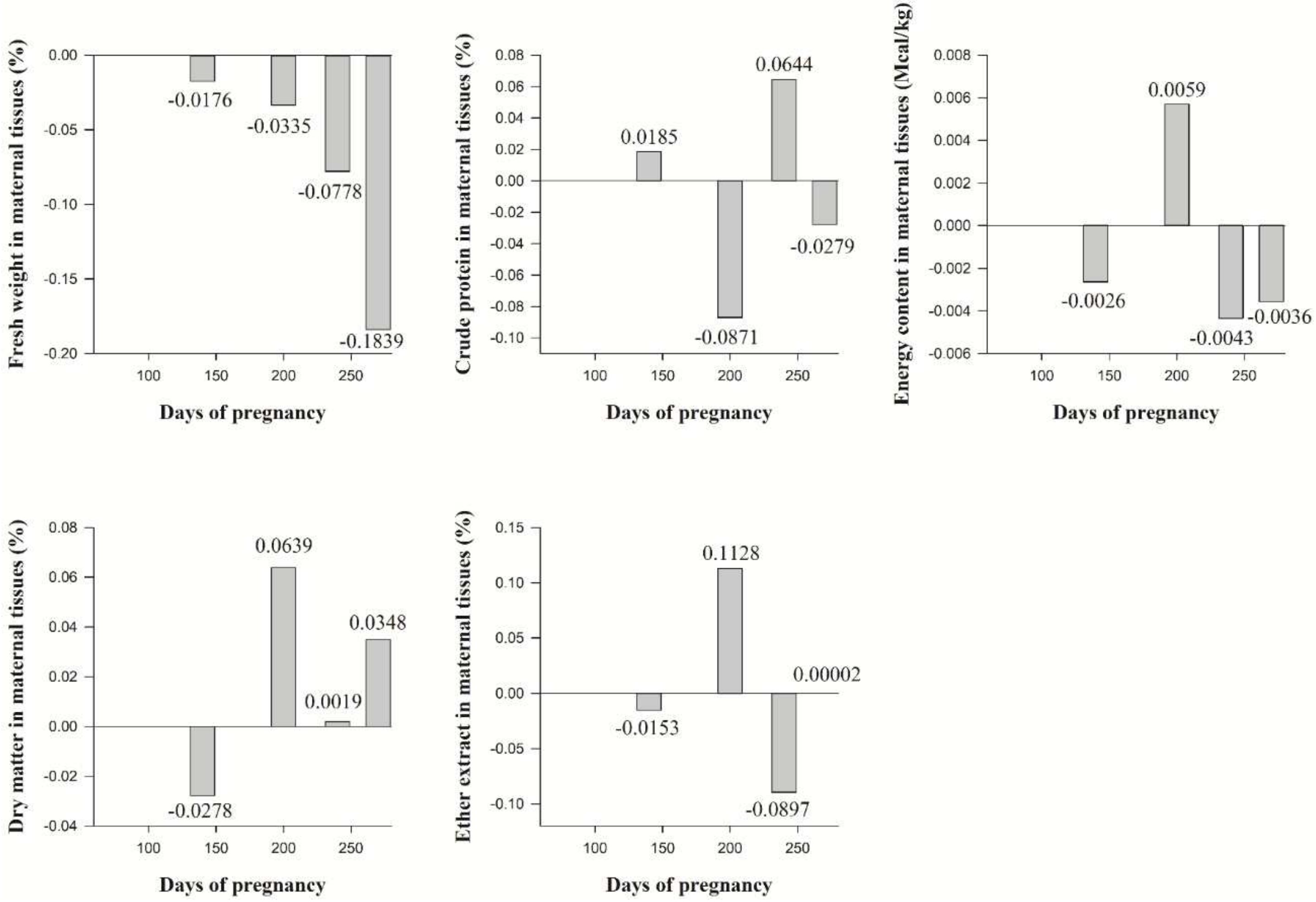
1- Uterus
2- Placenta
3- Fetus
4- Fetal fluids
5- Mammary gland

Gravid uterus = 1+2+3+4

B

Cow	**Carcass composition**	Bones and meat cuts from half carcass
	Non-carcass composition	Organs, stomachs, intestines, head, tail, hooves, trimmings, hide and blood.
	Maternal tissue	Sum of carcass and non-carcass components
	Mammary gland	The mammary gland with its skin, parenchimal, adipose tissue and colostrum.
	Gravid uteurus	The sum of uterus, placenta, fetus and fetal fluids.

Sguizzato, figure 4.



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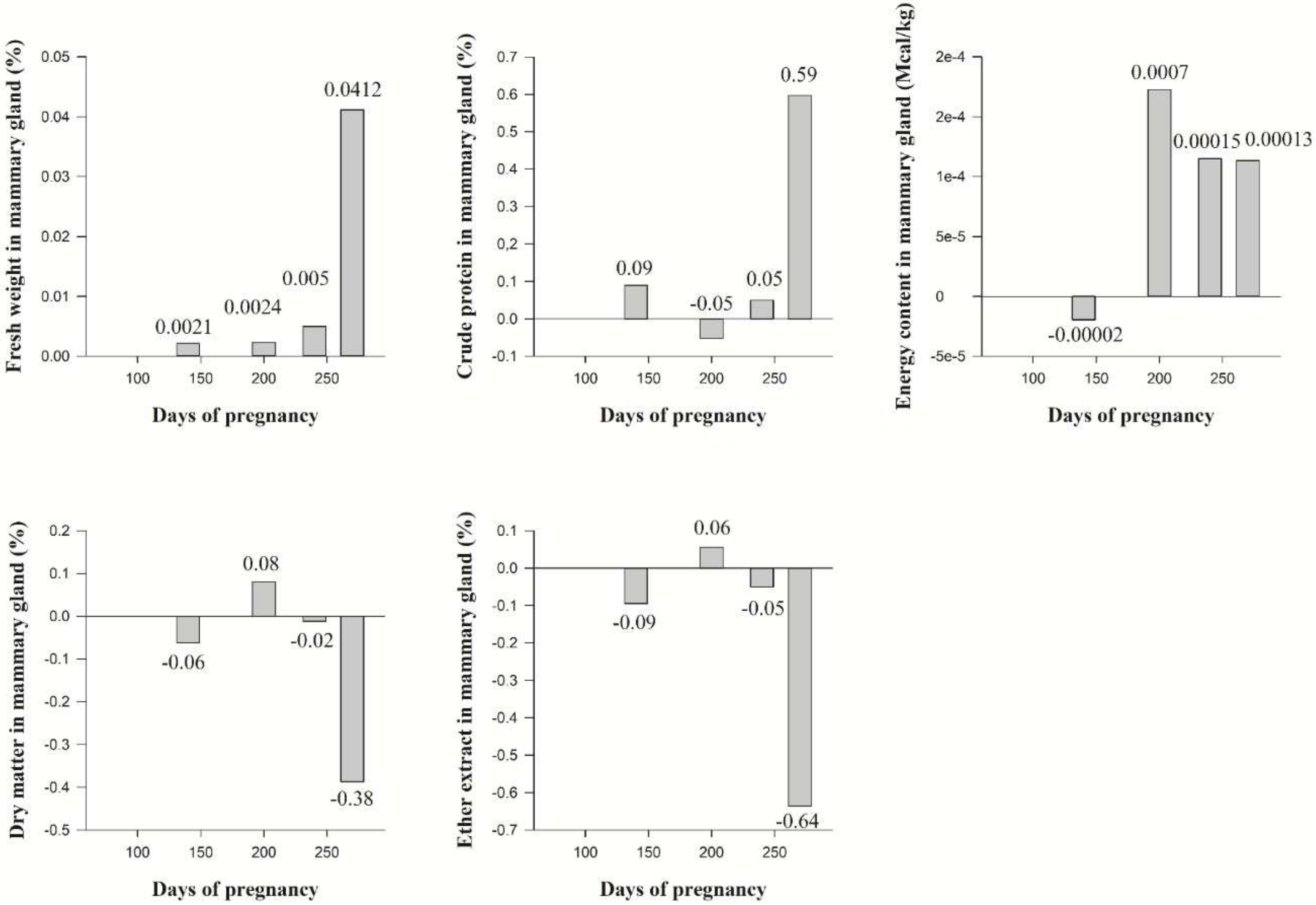

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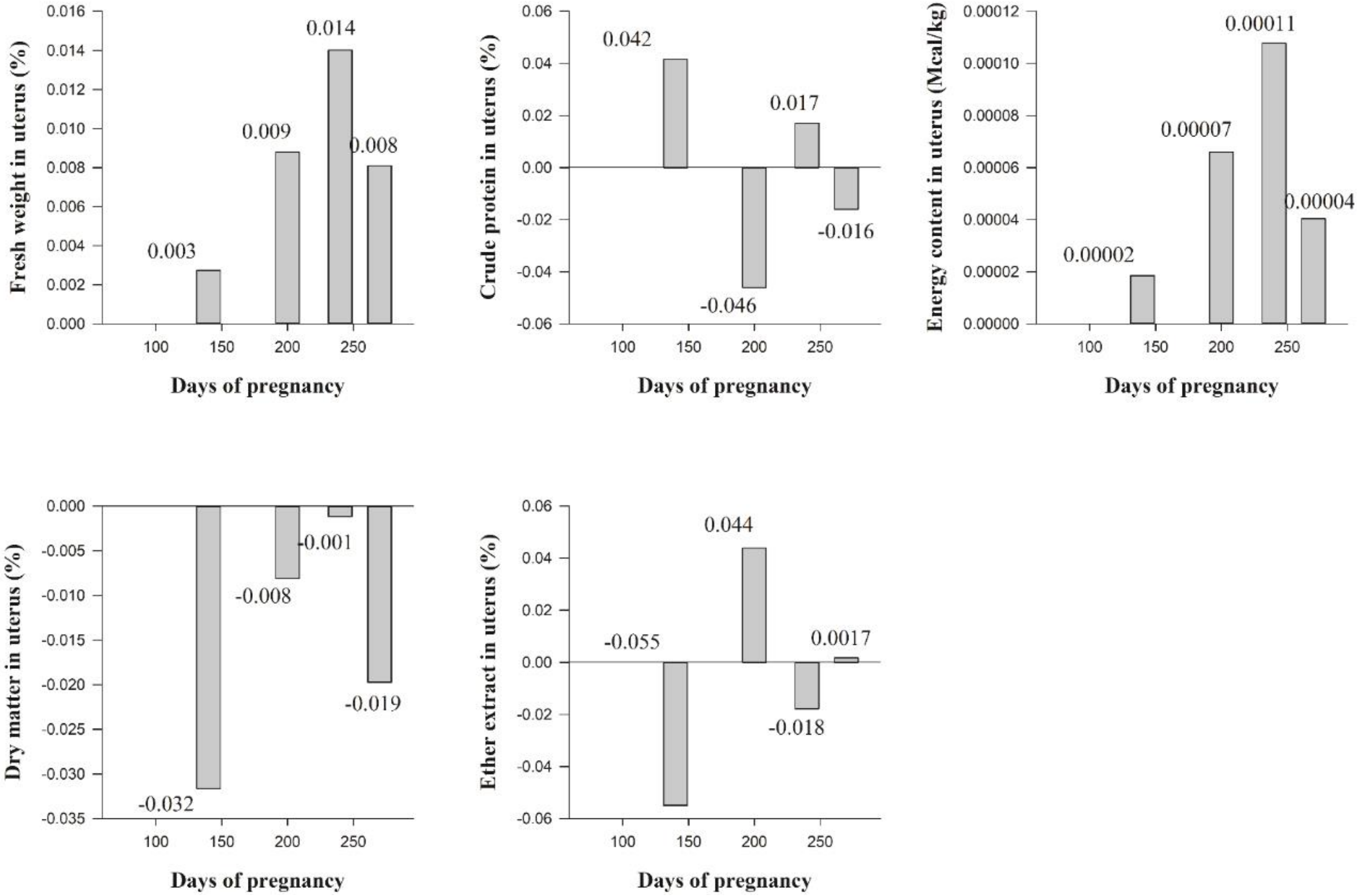

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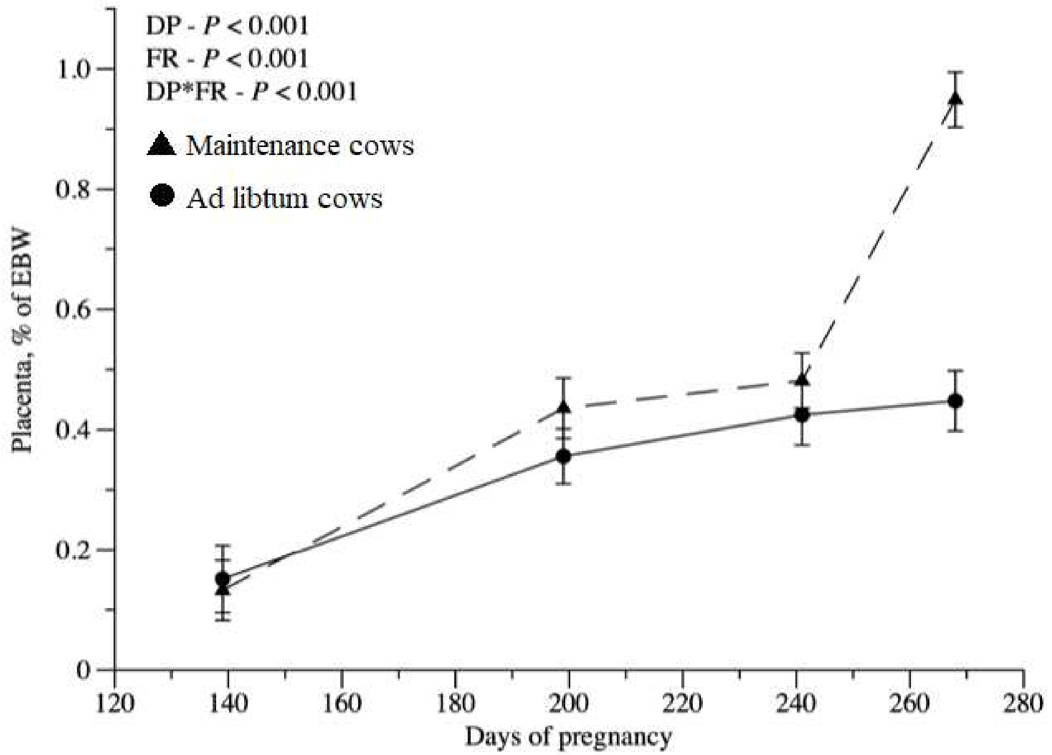

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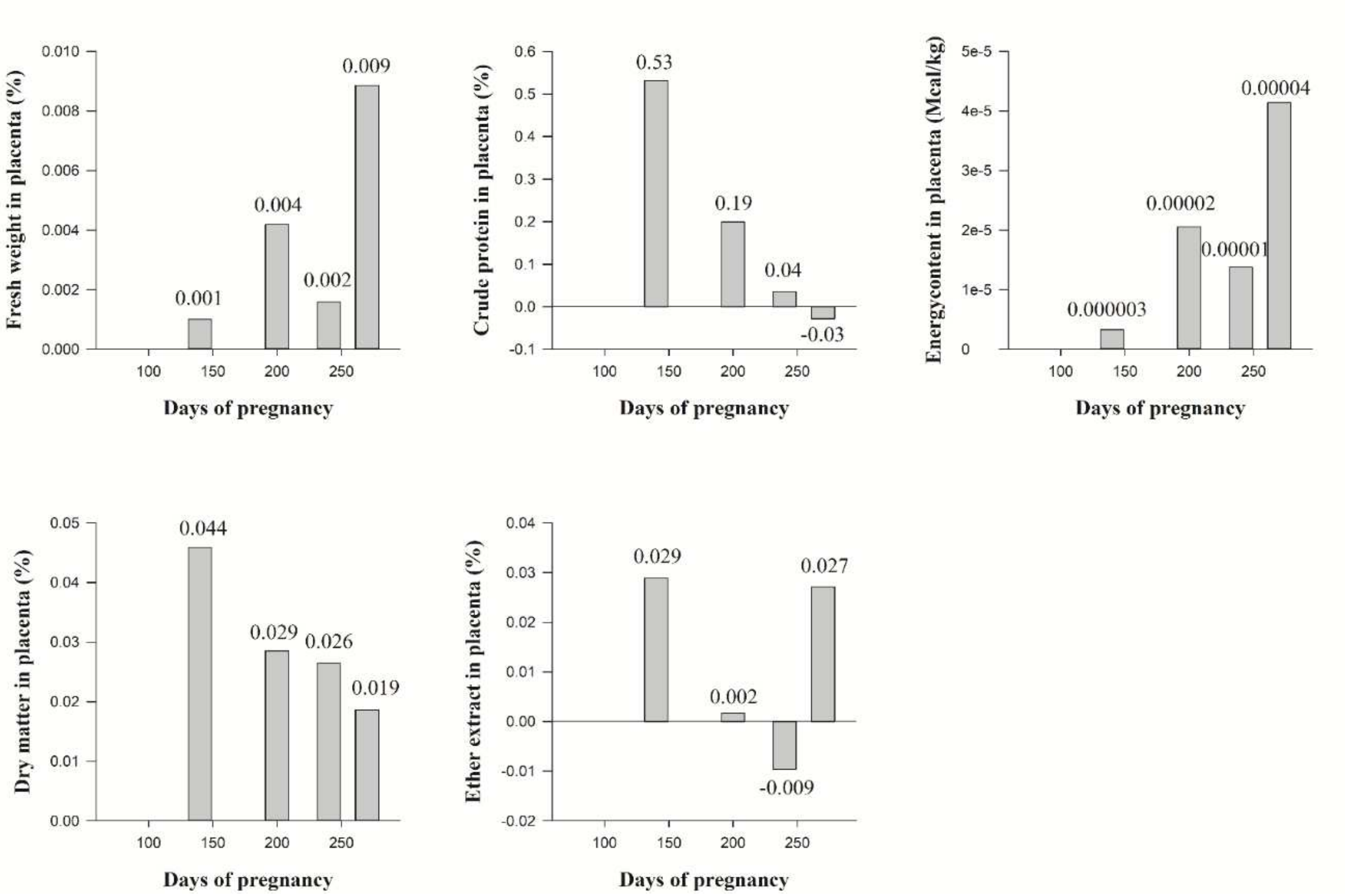

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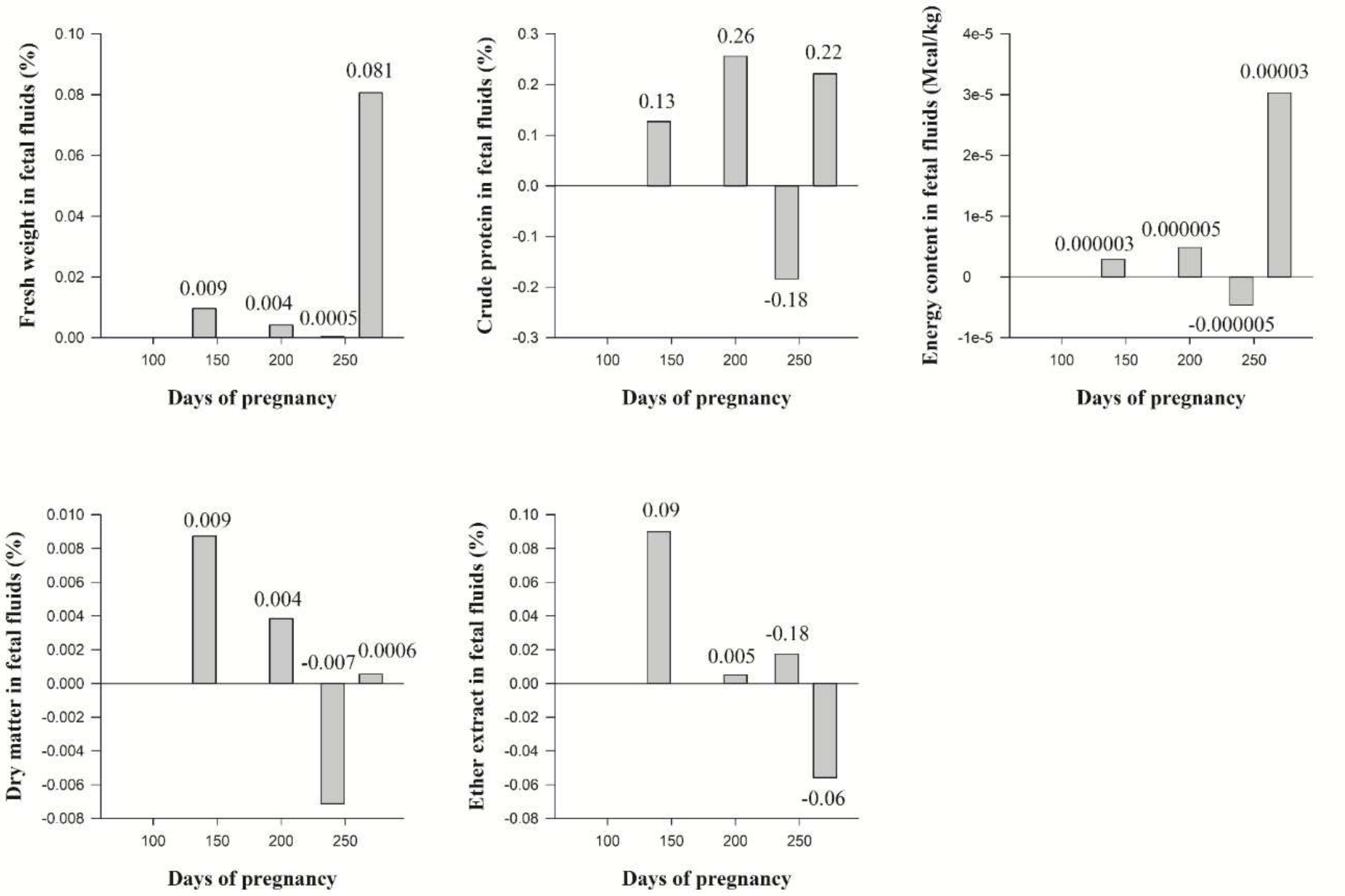

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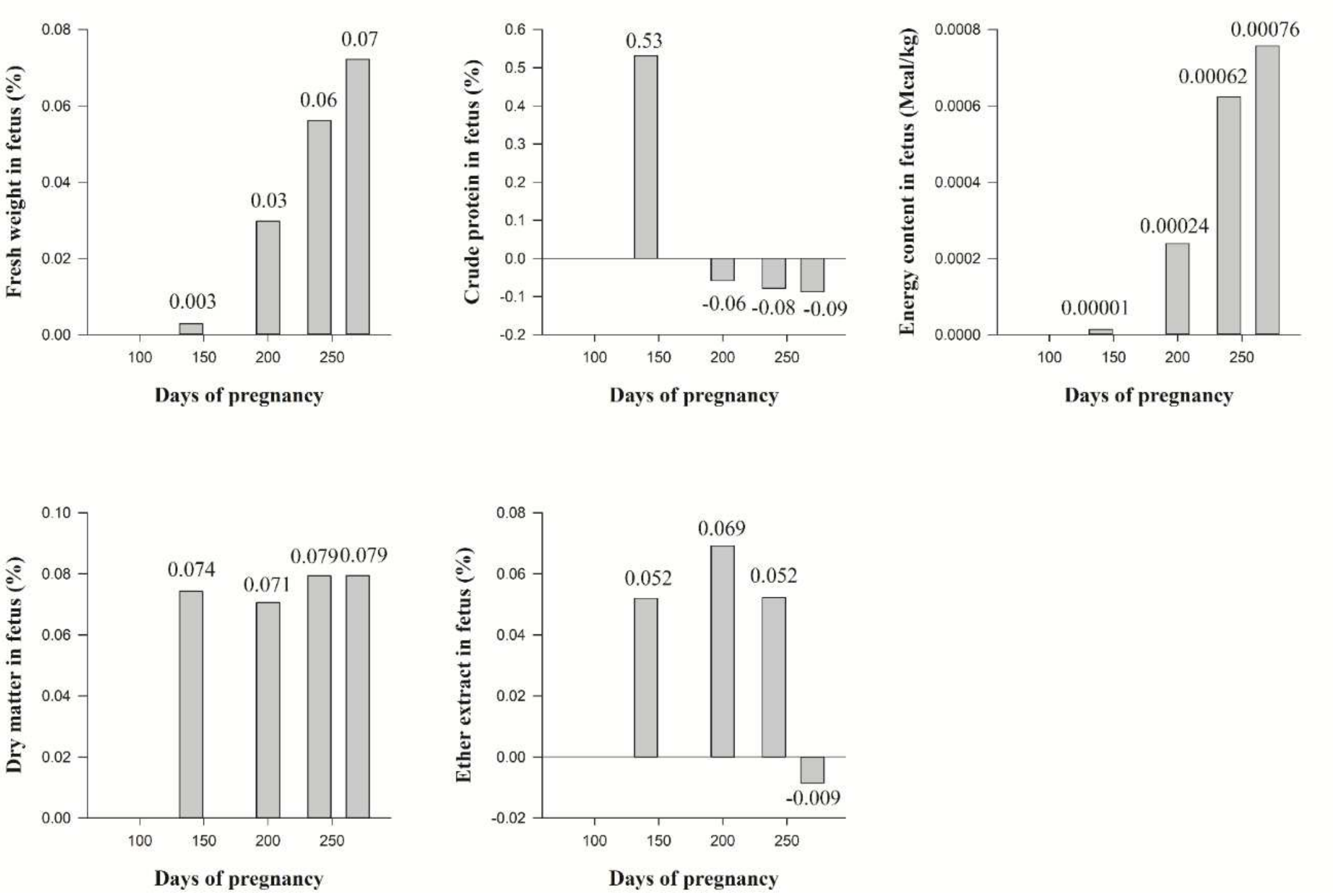

Sguizzato, figure 9.

CHAPTER 2

Energy requirements for pregnant crossbred Holstein × Gyr cows[1]

[1] Manuscript written to be submitted to the *Journal of Dairy Science*

ABSTRACT

This study aimed to estimate energy requirements of pregnant crossbred Holstein × Gyr cows. Differing planes of nutrition were established by two feeding regimes (**FR**): ad libitum or maintenance. Sixty-two nonlactating multiparous Holstein × Gyr cows with average body weight (**BW**) of 480 ± 10.1 kg and an age of 5 ± 0.5 years were used. Cows were divided into three groups: pregnant cows (n = 44), non-pregnant cows (n = 12), and baseline reference cows (n = 6). The 56 pregnant and non-pregnant cows were randomly allocated into FR: ad libitum (**AL**) or maintenance (**MA**). Cows fed at MA received 1.15% of their BW on a dry matter (**DM**) basis daily. Cows were fed corn silage and a concentrate-based diet at a ratio of 93:7 on a DM basis. Baseline reference cows were slaughtered at the beginning of the experimental period to estimate empty body weight (**EBW**) and composition. To evaluate the effects of days of pregnancy (**DP**), pregnant and non-pregnant animals were slaughtered at day 140, 200, 240, and (pregnant cows only) 270. Requirements for maintenance did differ between pregnant and non-pregnant cows, thus two equations were developed. Net energy (**NE**) and metabolizable energy (**ME**) requirements for maintenance of non-pregnant cows estimated in this study were 81 kcal/EBW$^{0.75}$/d and 131 kcal/EBW$^{0.75}$/d, respectively. The efficiency of use of ME for maintenance of non-pregnant cows was 61.8%. Net energy and ME for maintenance of pregnant cows were 85 kcal/EBW$^{0.75}$/d and 136 kcal/EBW$^{0.75}$/d, respectively. Efficiency of use of ME for maintenance of pregnant cows was 62.5%.

Estimates of net energy for gain ($\mathbf{NE_g}$) were different using non-pregnant and pregnancy cows' data, thus non-pregnant cows' requirements are recommended. The efficiency of use of ME for gain was 41.9%. Energy requirements for pregnancy were estimated fitting energy in gestation components (Mcal) in function of DP using a non-linear regression. The efficiency of use of ME for pregnancy was 15%. Furthermore, net energy for pregnancy was statistically significantly different from zero from day 70 of pregnancy. In conclusion, NE and ME for maintenance of non-pregnant Holstein × Gyr cows are different from pregnant cows. Regarding NE_g, we recommend estimating it only with data from non-pregnant animals. Furthermore, we believe that the proposed non-linear equations to estimate net energy requirements for pregnancy are more adequate for than current NRC equation, and should be recommended for Holstein × Gyr cows.

Key words: gravid uterus, Holstein × Gyr, nutrient requirements, pregnancy.

INTRODUCTION

In dairy cattle, the late pregnancy period is important to prepare the mammary gland for lactation. This is also a period of utmost importance for fetal development (Ferrell et al., 1976). Nevertheless, there are few studies to estimate energy requirements during pregnancy (Moe and Tyrell, 1972; Ferrell et al., 1976; Bell et al., 1995; BR-CORTE, 2016). Among available requirement systems (AFRC, 1993; NRC, 2001; CSIRO, 2007; INRA, 2007), the NRC (2001) is the most widely used for dairy cattle.

Energy requirements for pregnancy in the NRC (2001) were established according to results obtained by Bell et al. (1995) and the efficiency of use of metabolizable energy (**ME**) by the conceptus ($\boldsymbol{k_{preg}}$) was estimated by Ferrell et al. (1976).

According to Bell et al. (1995), the energy required for pregnancy is significant from day 190 of pregnancy, with linear accretion until day 279. These pregnancy requirements were obtained with cows from *Bos taurus* breeds. Thus, when evaluating requirements for animals from different breeds, such as crossbreds *Bos taurus* × *Bos indicus* (Holstein × Gyr), there is no clear evidence that energy requirements for pregnancy would be the same.

The Brazilian dairy herd is composed of approximately 70% of Holstein × Gyr animals (Ruas et al., 2011; Carvalho et al., 2018). This crossbred has greater milk production than the Gyr breed itself. This greater milk production is likely a result from its adaptability to tropical climate (Carvalho et al., 2018). In a meta-analysis, Oliveira (2015) found lower maintenance requirements and lower efficiency for milk production of crossbred *Bos taurus* x *Bos indicus*, compared to *Bos taurus* breeds. Nevertheless, to our knowledge, no quantitative data are available on nutrient requirements of pregnant crossbred Holstein × Gyr cows. In addition, no studies were found regarding requirements for gain of Holstein × Gyr cows.

Therefore, studies evaluating if nutrient requirements for pregnant Holstein × Gyr cows differ from requirements for *Bos taurus* breeds are warranted. Thus, we hypothesized that energy requirements estimated for pregnant crossbred Holstein × Gyr cows might differ from what is predicted by the NRC system based on Bell et al. (1995) and on ME efficiency of gravid uterus of Ferrell et al. (1976). For this reason, the aim of this study was to estimate energy requirements of maintenance, pregnancy, and gain of pregnant crossbred Holstein × Gyr cows.

MATERIAL AND METODS

This study was conducted at Universidade Federal de Viçosa (Viçosa, MG, Brazil) following the standard procedures for humane animal care and handling according to the university's guidelines (CEUAP/UFV – 47/2012).

Animals and Management

Data used in the present analysis were obtained from a previous study conducted by Rotta et al. (2015a, b; c). Briefly, sixty-two Holstein × Gyr cows were used. They were divided into 3 groups: pregnant (n = 44), non-pregnant (n = 12) and baseline (n = 6). However, one abortion was verified in a cow from the ML treatment at 140 d. Thus, data from 43 cows were used for analyses, and 5 ML cows were evaluated at 140 d of gestation. Baseline animals were slaughtered at day 0. Both pregnant and non-pregnant cows received two different feeding regimes (**FR**): *ad libitum* (**AD**) and maintenance (**MA**) (1.15% of body weight (**BW**)). These 43 Holstein × Gyr cows were slaughtered at four different days of pregnancy (**DP**): day 140, 200, 240 and 270. The 12 remaining cows (non-pregnant cows) were slaughtered at day 200, 240 and 270 in feedlot (Figure 1).

Cows were housed in 30 m^2 individual pens, of which 8 m^2 was covered with concrete floors and equipped with individual feed bunks and an automatic water system. They were fed corn silage and a concentrate-based diet at a ratio of 93:7 on a DM basis as a TMR twice daily. The amounts of corn silage, concentrate, and orts were recorded daily. All cows had *ad libitum* access to water and in order to allow *ad libitum* access to feed, its delivery was adjusted to approximately 5% orts daily on an as-fed basis.

Slaughtering Procedures and Sampling

Cows were slaughtered by a captive bolt stunner, followed by exsanguination.

Right after exsanguination, the gravid uterus was immediately collected and weighted. It was sectioned in cervix height and then dissected in fetus, fetal membranes, uterus and fetal fluids, in a way that the weight of fetal fluids was obtained by difference between pregnant uterus and the other sectioned parts. Fetus, fetal membranes, and uterus were ground and sampled individually. The mammary gland was also sectioned and entirely ground (Figure 2). Then, a homogenized sample was created with uterus and fetal membranes. Samples from gravid uterus and mammary gland were maintained at -80ºC until further chemical analyses.

The carcass of each animal was divided into two half carcasses. They were weighted to determine carcass hot yield, then allocated in a cold chamber at 4ºC, during 24h. Posteriorly, the carcasses were weighted to determine cold carcass yield. In addition, to compose the non-carcass sample, the four stomachs, small and large intestines were washed after slaughter and added to internal organs, head, tail, hooves, trimmings, leather, and blood. All components were ground and homogenized, and then a sample of each carcass and non-carcass was taken for further analyses and finally compose cow's tissue sample (Figure 2).

There were six periods of spot fecal collections, for evaluation of apparent total-tract digestibility, with each period lasting 28 d. Feces from all cows were collected during the last 5 d of each 28-d period. Fecal collections were performed at 0600, 0900, 1200, 1500, and 1800 h on d 1, 2, 3, 4, and 5, respectively. A composite sample was obtained per collection period for each cow by utilizing 15 g of the dried and ground sample per collection time (Rotta et al., 2015a, b; c).

Laboratorial Analyses

Samples of carcass, non-carcass, mammary gland, uterus, placenta, fetal fluids, fetus and feces were analyzed for DM (AOAC, 2000; method 934.01), CP (AOAC, 2000; method 981.10), and ether extract (**EE**) (AOAC, 2006; method 945.16). Energy content was estimated based on protein and ether extract contents, as proposed by ARC (1980).

Fecal DM excretion was estimated by the internal marker technic (Lippke et al., 1986), where indigestible neutral detergent fiber was the internal marker. Indigestible neutral detergent fiber content from feces, feeds, and orts were quantified in triplicate and obtained by in situ incubation procedures. The bags were incubated for 288 h (Valente et al., 2011) in the rumen of 2 cannulated bulls fed a diet in a 50:50 ratio on a DM basis at maintenance level. More details about management and laboratorial analyzes are available in Rotta et al. (2015a, b, c).

Calculations and Estimated Equations

Nonfiber carbohydrates (**NFC**) content was calculated according to Detmann and Valadares Filho (2010):

$$NFC = 100 - [(\%\,CP - \%CPUrea + \%Urea) + \%NDF + \%EE + \%\,CA] \quad [1]$$

Where: %CPurea is the CP from urea (urea-N × 6.25); and %CA is crude ash content, and all values are in % of DM.

The digestible energy (**DE**) of the diet was obtained by multiplying the digestible fraction of each nutrient by its caloric value (ARC, 1980):

$$DE = 5.6 \times dCP + 9.4 \times dEE + 4.2 \times dNDF + 4.2 \times dNFC \quad [2]$$

Where: DE = digestible energy (Mcal/d); dCP = digestible crude protein (kg/d); dEE = digestible ether extract (kg/d); dNDF = digestible neutral detergent fiber (kg/d) and dNFC = digestible nonfiber carbohydrates (kg/d).

Metabolizable energy was obtained by the equation from NRC (2000):

$$ME = DE \times 0.82 \qquad [3]$$

Where ME = metabolizable energy (Mcal/kg DM) and DE = digestible energy (Mcal/kg DM).

Empty body weight (**EBW**) of cows is composed by carcass, non-carcass, mammary gland and uterus. For pregnant cows, in order to estimate uterus and mammary gland components exclusively due to pregnancy, uterus and mammary gland components were estimated as if they were open. The EBW energy content for both non-pregnant and pregnant cows were obtained from the body contents of protein and fat and their respective caloric equivalents of 5.7 and 9.5 Mcal/kg (ARC, 1980).

Heat production was calculated as the difference between ME intake and retained energy (**RE**), where RE was calculated as the difference between the initial and the final total body energy content. The net energy requirement for maintenance (**NE_m**) was assumed to be the intercept (β_0) of the exponential regression between MEI and heat production (**HP**) (Equation 4), as proposed by Ferrell and Jenkins (1998).

$$HP = \beta_0 \times e^{\beta_1 \times MEI} \qquad [4]$$

Where: HP = heat production (Mcal/$EBW^{0.75}$/d), MEI = metabolizable energy intake (Mcal/$EBW^{0.75}$/d), and β_0 and β_1 are the equation parameters.

For all comparisons between non-pregnant and pregnant animals, and among pregnancy groups, for the purpose of statistical tests, animals slaughter between 137 and 144 days of pregnancy were included in the model as 140 days. Animals slaughtered between 196 and 201 days were included in the model as 200 days, animals slaughtered between 236 and 247 days were included in the model as 240 days. Lastly, animals slaughtered between 266 and 270 days of pregnancy were included in the model as 270 days. In order to estimate NE_m for non-pregnant and pregnant cows separately, we fitted two models. The first model was estimated using non-pregnant cows only, and the second one was estimated using pregnant cows at d 140, 200, 240 and 270. The effect of days of pregnancy was tested on both parameters of Equation 4.

Metabolizable energy requirements for maintenance (**ME$_m$** in Mcal/EBW$^{0.75}$/d) were estimated by the iterative method, as the point where MEI equals HP (i.e., the point at which there is no energy retention in the body). In addition, the efficiency of use of ME for maintenance (**k_m**) was estimated by the ratio between NE and ME requirements for maintenance. Separate estimates of ME$_m$ for non-pregnant and pregnant cows were obtained.

Requirements of net energy for gain of non-pregnant animals were estimated as retained energy according to the equation proposed by Garrett (1980).

$$NEg = \beta_0 \times EBW^{0.75} \times EBG^{\beta_1} \qquad [5]$$

Where: NE$_g$ = net energy for gain (Mcal/d), EBW$^{0.75}$ = metabolic empty body weight (kg), EBG = empty body gain (kg/d) and β_0 and β_1 are equation parameters.

Requirements of net energy for gain in pregnant cows were considered as the amount of retained energy in maternal tissue (**MT;** which consists of EBW minus the gravid uterus minus the mammary gland) of pregnant animals. Retained energy in **MT** of pregnant cows (**RE$_{MT}$**) was estimated by a non-linear regression in function of MT composition (kg), where only carcass and non-carcass weight were considered (Figure 2), and gain of maternal tissue (**GMT**, in kg/d), Equation 6. Requirements for non-maternal tissues (mammary gland and gravid uterus) in pregnant cows were calculated separately.

$$RE_{MT} = \beta_0 \times MT^{0.75} \times GMT^{\beta_1} \qquad [6]$$

Where: RE$_{MT}$ = Retained energy in maternal tissue of pregnant cows (Mcal/d),

$MT^{0.75}$ = average metabolic maternal tissue (kg), GMT = gain of maternal tissue (kg/d) and β_0 and β_1 are equation parameters. Average maternal tissue was considered the average of initial MT (**iMT**) and final MT (**fMT**). A linear regression considering MT from animals of the baseline group in function of EBW was used to estimate iMT.

To estimate energy requirements for pregnancy, the balance of pregnancy components and days of pregnancy was considered. Balance of pregnancy components was calculated as the sum of the difference between final and initial energy content of the gravid uterus and the difference between final and initial energy content of the udder in Mcal (**GEST**). To predict initial GEST, a linear regression was estimated in function of final GEST (Mcal) and final EBW, with baseline group and non-pregnant cows.

Three initial models were selected to fit GEST weight and composition in function of DP: one linear and two non-linear models (quadratic, simple exponential and double exponential, respectively) (Equation 7, 8, and 9).

$$Quadratic\ model = \beta_0 + \beta_1 DP + \beta_2 DP \qquad [7]$$

$$Simple\ exponential\ model = \beta_0 \times exp^{(\beta_1 \times\ DP)} \qquad [8]$$

$$Double\ exponential\ model = \beta_0 \times exp^{exp^{(\beta_1 \times\ DP)}} \qquad [9]$$

Where: DP = is the days of pregnancy and β_0 and $\beta 1$ are equation parameters.

To evaluate the best fit, the AIC was used and equation 8 presented the lowest AIC (303.9, *data not shown*). The first derivate of Equation 8 was assumed to be the net energy for pregnancy (**NE_{preg}**). In addition, an adjustment in function of calf body weight (**CBW**) was added to the model as proposed by NRC (2001).

The efficiency of use of ME for gain of non-pregnant cows ($\boldsymbol{k_g}$) was estimated according to Marcondes et al. (2013), considering only MEI above maintenance. The

$\boldsymbol{k_g}$ was assumed to be the slope (β_1) of the regression of RE in function of MEI for gain (MEI_g; calculated as MEI – ME_m).

$$RE = \beta_0 + \beta_1 \times MEIg \quad [10]$$

Where: RE = retained energy (Mcal/kgEBW$^{0.75}$/d), MEI_g = metabolizable energy intake for gain (Mcal/kgEBW$^{0.75}$/d) and β_0 and β_1 are equation parameters.

The efficiency of use of ME for pregnancy ($\boldsymbol{k_{preg}}$) was estimated by iterative method using Equation 11.

$$\Delta = MEI - \left(\frac{NE_m}{k_m} + \frac{RE_{MT}}{k_g} + \frac{GEST}{k_{preg}}\right) \quad [11]$$

Where: MEI = daily metabolizable energy intake, NE_m = daily net energy requirement for maintenance estimated in this study, RE_{MT} = daily retained energy in maternal tissue, GEST = daily retained energy in the gravid uterus and the udder, k_m = efficiency of use of metabolizable energy for maintenance estimated in this study, k_g = efficiency of use of metabolizable energy for gain and k_{preg} = efficiency of use of metabolizable energy for pregnancy. The iteration was performed aiming an average Δ of zero. The parameters NE_m, k_m, RE_{MT}, k_g and GEST were already calculated. Thus, we estimated only the k_{preg} by iteration.

Requirements of metabolizable energy for gain (**ME_g**) of pregnant and non-pregnant cows were estimated by the ratio between NE_g and k_g. Requirements of metabolizable energy for pregnancy (**ME_{preg}**) were estimated by the ratio between NE_{preg} and k_{preg}.

Statistical Analyses

The model used to estimate maintenance requirements was fit using PROC NLMIXED of SAS (version 9.4; SAS Institute, Inc. Cary, NC). The effect of physiological condition (pregnant and non-pregnant) was tested on both parameters,

β_0 and β_1. Models to estimate requirements for gain were evaluated using PROC NLMIXED of SAS (version 9.4; SAS Institute, Inc. Cary, NC). The effect of physiological condition was also tested on β_0 and β_1. Regarding requirements for pregnancy, effects of FR were tested on both parameters β_0 and $\beta 1$ of Equation 8 using PROC NLMIXED of SAS (version 9.4; SAS Institute, Inc. Cary, NC).

For all statistical analyses, the student t test was used to compare FR, and all significances were declared when P < 0.05.

RESULTS AND DISCUSSION

Net and Metabolizable Energy Requirements for Maintenance

When evaluating the effect of gestation days on the coefficients of the model to predict the net energy required for maintenance, we observed a difference between non-pregnant and pregnant cows on both parameters β_0 and β_1. The difference found between d 0 and 140 (P = 0.010 and P = 0.025, respectively) indicated that, during pregnancy, Holstein × Gyr cows may present a distinct NE_m (Figure 3). No differences were observed from d 140 to d 270 of gestation (P > 0.05). Thus, two equations were considered, one for each condition (non-pregnant, Equation 12; and pregnant, Equation 13).

$$HP_{non-pregnant} = 0.081 \pm 0.0045 \times e^{3.600 \pm 0.2259 \times MEI} \quad [12]$$

($R^2 = 0.9676$; RMSE = 0.00004)

$$HP_{pregnant} = 0.085 \pm 0.0032 \times e^{3.442 \pm 0.1455 \times MEI} \quad [13]$$

($R^2 = 0.9279$; RMSE = 0.0001)

Where: HP and MEI are in $Mcal/EBW^{0.75}/d$.

The estimated value of NE_m in this study for non-pregnant cows was 81 $kcal/EBW^{0.75}/d$ or 74 $kcal/BW^{0.75}/d$. Therefore, the value obtained is approximately

8% lower than the NE_m value from NRC (2001), which is 80 kcal/$BW^{0.75}$/d (Figure 4 A). The NE_m requirements for pregnant cows were 85 kcal/$EBW^{0.75}$/d or 79 kcal/$BW^{0.75}$/d. This value is closer to the NE_m suggested by the NRC (2001). Lage (2011) found values of 77 and 92 kcal/$BW^{0.75}$/d of NE_m for non-pregnant Gyr and crossbred Holstein × Gyr heifers, respectively. Estimations for energy requirements by Lage (2011) were obtained by the indirect calorimetry technique. Furthermore, the BR-CORTE (2016) considers NE_m for pregnant and non-pregnant Nellore cows of 86 kcal/$EBW^{0.75}$/d, a value close to the estimate of pregnant cows in this study.

Metabolizable energy for maintenance was estimated by the iterative method, as the point where HP equals MEI. The ME_m for non-pregnant cows was 131 ± 2.49 kcal/$EBW^{0.75}$/d or 118 kcal/$BW^{0.75}$/d (Figure 4 B). The efficiency of use of ME for maintenance (k_m) for non-pregnant cows was 61.8%. However, for pregnant cows, ME_m was greater than non-pregnant cows, 136 ± 1.72 kcal/$EBW^{0.75}$/d or 126 kcal/$BW^{0.75}$/d. The k_m for pregnant cows was 62.5%.

Solis et al. (1988), compared energy requirements of five breeds, three for beef production and two for milk production. In their study, they found a ME_m of 119 kcal/$BW^{0.75}$/d for Holstein cows. The NRC (2001) considers a k_m of 64% and ME_m is about 125 kcal/$BW^{0.75}$/d. Lage (2011) also estimated ME_m for heifers and found greater values, 120 and 146 kcal/$BW^{0.75}$/d for Gyr and Holstein × Gyr crossbred, respectively. The BR-CORTE (2016) considers as ME_m a value of 120 kcal/$EBW^{0.75}$/d for non-pregnant and pregnant Nellore cows, with an accretion of 8.5% in requirements for ME_m when animals are raised on pasture.

Our estimates of NE_m and ME_m for non-pregnant and pregnant cows presented a difference, on average, of 5%. However, for a greater MEI, HP becomes closer for cows in both physiological states. Moreover, non-pregnant cows showed ME_m values close to those of Solis et al. (1988) and Lage (2011) (considering the Gyr heifers' results from the last author). In contrast, pregnant cows had NE_m and ME_m much closer to NRC (2001) requirements, indicating similarity among these recommendations, regarding maintenance requirements.

Solis et al. (1988) also found distinct differences for maintenance requirements among beef and milk breeds. The NASEM (2016) and BR-CORTE (2016) also suggest different requirements for *Bos taurus* and *Bos indicus* breeds. A possible explanation to these lower results obtained for non-pregnant cows may be related to the size of internal organs and amount of visceral fat (Solis et al., 1988; Ferrell and Jenkins, 1998; Oliveira, 2015), which may be smaller for crossbred Hostein × Gyr cows. Even though a small proportion of BW is composed by internal organs, they contribute to high metabolic rates (Ferrell and Jenkins, 1998), accounting for 40 to 50% of energy requirements in ruminants (Lage, 2011), which results in an increase in HP.

Furthermore, during pregnancy, the gravid uterus and the mammary gland accounts for a greater increase in HP. Considering the increase in NE_m and ME_m for pregnant cows, it suggests an increase in HP produced by the gravid uterus and/or developing mammary gland. Our calculations account exclusively the increase in gravid uterus and mammary gland due to pregnancy; and according to our results and observations, there may be a greater increase in HP caused by the gravid uterus than caused by the mammary gland, especially because the fetus uses mainly amino acids as source of energy, which is linked to a greater HP when compared with carbohydrates metabolism.

Net and Metabolizable Energy Gain

Energy requirements for gain were estimated using data from non-pregnant (Equation 14) and pregnant cows (Equation 15).

$$NE_g = 0.0625 \pm 0.0425 \times EBW^{0.75} \times EBG^{0.9206 \pm 0.0937} \quad [14]$$

($R^2 = 0.906$; RMSE = 0.0095)

Where: NE_g = net energy for gain (Mcal/d), $EBW^{0.75}$ = metabolic empty body weight (kg), EBG = empty body gain (kg/d).

$$RE_{MT} = 0.0600 \pm 0.0022 \times MT^{0.75} \times GMT^{0.6562 \pm 0.0725} \quad [15]$$

($R^2 = 0.879$; RMSE = 0.8192)

Where: RE_{MT} = retained energy in maternal tissue of pregnant cows (Mcal/d), $MT^{0.75}$ = average metabolic maternal tissue (kg), GMT = gain of maternal tissue (kg/d).

The pattern of energy deposition in EBG and MT among non-pregnant and pregnant cows is similar (Figure 5). However, we could not identify the reason why the discrepancy occurred between the β_1 parameters in both equations for non-pregnant (Equation 14) and pregnant cows (Equation 15). The β_1 parameter obtained in equation 14 represents the variation on gain composition according to the amount of retained energy per day. Therefore, according to the estimated value in this study, for greater rates of gain (at the same BW), a greater proportion of protein will be deposited, instead of energy as fat. The β_1 coefficient of EBG (0.9206), for non-pregnant cows, corroborates the literature (Rotta et al., 2013; Neves et al., 2016; Silva et al., 2018), and a coefficient of 0.6562 (Equation 15) suggests a high proportion of protein in total gain during pregnancy. Moreover, pregnant cows may have had greater rates of protein turnover, indicating the difference observed for the β_1 parameter. Based on the β_1 coefficient obtained for non-pregnant animals when compared to that obtained for pregnant cows (Equation 15) we suggest the use of

Equation 14 for both pregnant and non-pregnant cows when estimating NE_g. Future studies should focus efforts on body composition changes during pregnancy to certify that this is indeed happening. Nevertheless, in this study, pregnancy did not affect CP or EE in both carcass and non-carcass components (Sguizzato et al., 2019, *unpublished data*)

The efficiency of use of ME for gain was estimated according to Marcondes et al. (2013) (Eq. 10), using only data from non-pregnant cows. The β_1 was considered as the k_g, which was 41.9%.

$$RE = -0.0558 \pm 0.0127 + 0.4189 \pm 0.0542 \times MEI_g \qquad [16]$$

($R^2 = 0.869$; RMSE = 0.0001)

Where: RE = retained energy ($Mcal/kgEBW^{0.75}/d$) and MEI_g = metabolizable energy intake for gain ($Mcal/kgEBW^{0.75}/d$).

The efficiency of use of ME for gain estimated in this study is lower than that considered by the NRC (2001). In the present study, k_g was 41.9%, approximately 30% lower than the value used by NRC (2001), which is 60% for non-lactating cows (Moe et al., 1970), while the NRC (2001) suggests a k_g of 75% (Moe et al., 1971) for lactating cows. According to NRC (2001), lactating animals are substantially more efficient than growing animals, and a higher k_g is recommended for animals that are not considerably changing the body composition (Moe et al., 1971). The BR-CORTE (2016) also found greater k_g for mature beef *Bos inidicus* cows (53%) than the one we observed for Holstein × Gyr cows. However, our data does not support a k_g ranging from 53 to 60%. It is possible that pregnant animals, especially those close to parturition, might have their body composition altered (Kenéz et al., 2015), which does not support the k_g suggested by NRC (2001). According to the ARC (1980), a high metabolizability (ME/GE ratio) coincides with a greater k_g; however, in our study the metabolizability was 0.55 ± 0.01, which might explain the lower k_g observed.

Nonetheless, Oss et al. (2017); Silva et al. (2018); Castro et al. (UFV, Viçosa, MG, marcelo.duarte@ufv.br), evaluating energy requirements of Holstein × Gyr bulls and heifers, reported a k_g of 30.5% (average BW of 235 kg), 40.8% (average BW of 218 ± 36.5 kg) and 32.0% (average BW of 102.2 ± 3.4), respectively. Therefore, our k_g was closer to values reported for growing animals, which have greater rates of energy deposited as protein than as fat. Moreover, the efficiency of gain proposed by BR-CORTE (2016) demonstrated a gain composition with greater proportion of energy deposited as fat in adult animals, as discussed above. Nonetheless, we recommend a k_g of 41.9% for Holstein × Gyr adult cows. We also suggest, based on our results and previous literature (Rotta et al., 2013; Gionbelli et al., 2015; Silva et al., 2018) that the k_g reported in NRC (2001) should be reviewed, because it might overestimate k_g in particular for transition cows. The efficiency of utilization of body store reserves for milk production in early lactation is markedly higher than the efficiency of utilizing dietary ME for tissue energy gain (Moe et al. 1971; Moraes et al. 2015). This event, associated with the replenishment of body lipids mobilized at early lactation, leads to a higher k_g than that of gain of body protein.

Net and Metabolizable Energy Requirements for Pregnancy

To estimate pregnancy requirements, the balance of pregnancy components (GEST) was used to estimate the RE related only to pregnancy. The same methodology was used by Ferrell et al. (1976), Bell et al. (1995). Lage (2015) and BR-CORTE (2016), however there were some particularities among them. The idea of GEST utilized in this study is similar to the one adopted by BR-CORTE (2016).

The GEST component is the accretion in udder, uterus, and all the other components of the gravid uterus due to pregnancy. Following the establishment of GEST, a non-linear regression was fit to estimate GEST in Mcal as a function of DP (Eq. 8, Figure 6 B). The first derivate of Eq. 8, adding a correction factor for expected calf body weight (NRC, 2001), was considered as NE_{preg}.

$$NEpreg = 0.02105 \pm 0.6475 \times exp^{(0.0141 \pm 0.0017 \times DP)} \times (expected\ CBW/35) \quad [17]$$

($R^2 = 0.741$; RMSE = 136.3)

Where: NE_{preg} = net energy for pregnancy (Mcal/d) (representing energy retained in the gravid uterus and mammary gland), DP = days of pregnancy, CBW = expected calf body weight (kg).

Expected CBW (35 kg) was obtained according to a database of approximately 1,800 crossbred Holstein × Gyr calves of the official control of EMBRAPA Dairy Cattle (*data not shown*). The NRC (2001) uses a linear regression equation to estimate NE_{preg} considering DP and CBW, and the fetus accounts for approximately 80% of uterine dry weight (Bell et al., 1995). However, non-linear regressions have been used to estimate animal's development allowing a greater representation of biological growth (Tedeschi et al., 2000).

Estimations of energy requirements for pregnant cows by a linear (NRC, 2001) and non-linear regression (our study) are illustrated in Figure 6. According to the results presented in Figure 6 A, NE_{preg} for crossbred Holstein × Gyr cows is lower than for purebred Holstein cows (NRC, 2001) until day 230 of pregnancy, approximately. After this period, estimated NE_{preg} surpass the requirements predicted by NRC (2001). Moreover, Figure 6 B shows the pattern of RE in the gravid uterus and mammary gland, with an equal pattern to NE_{preg} and the accretion, illustrated in Figure 6 B, allows a greater representation of energy requirements for pregnancy.

Our model shows greater similarity among biological growth models (Tedeschi et al., 2000) than do linear models (Bell et al., 1995). In addition, the NASEM (2016) and INRA (2007) also considers the use of a non-linear regression to estimate pregnancy requirements. Their values are much closer to ours when compared to NRC (2001). Figure 6 C shows ME_{preg} obtained from NRC (2001) equation and the equation from this study. As in NE_{preg} for crossbred Holstein × Gyr cows, ME_{preg} is lower than for purebred Holstein cows (NRC, 2001) until day 235 of pregnancy, approximately. After this period, estimated NE_{preg} surpass the requirements predicted by NRC (2001). Indicating greater increase in requirements for pregnancy after d 230.

Lage (2015) found NE_{preg}, represented by RE in gravid uterus and mammary gland for Holstein × Gyr cows of 2.70, 2.71, and 2.88 Mcal/d on days 180, 210 and 240 of pregnancy, respectively. Net energy requirements obtained by Lage (2015) in her study are closer to NRC (2001) when compared to our values. However, observing NE_{preg} obtained in our study, we found similar results to Ferrell et al. (1976) as they obtained retained energy for pregnancy (Table 1). This proximity of values for Holstein × Gyr cows and Hereford heifers could be principally due to the exponential models used to estimate energy content in the gravid uterus. Net energy for pregnancy for beef cattle (NASEM, 2016) is estimated by a similar non-linear equation from Ferrell et al. (1976).

The retained energy in the gravid uterus and mammary gland through DP may be observed in Figure 7. There is a greater increase of retained energy in the gravid uterus than in mammary gland. From d 140 to d 240 the amount of energy retained in the gravid uterus is greater than from d 240 to d 270. It may be indicative of uterus growth to support fetus development during the final period of pregnancy. Changes in mammary gland also account for pregnancy requirements. However, changes are smaller when compared to the gravid uterus. This pattern of energy deposition may occur due to the fact that the involution of the mammary gland itself, after lactation, is smaller in proportion when compared to uterus involution. The uterus grows to a size enough to support the size of a calf and after birth, it recovers its initial size.

Efficiency of use of ME for pregnancy was estimated by the iterative method. The obtained value for k_{preg} was 15 ± 0.48 %, which very close to values obtained by Ferrell et al. (1976), 14%; Lage (2015), 12.5% and BR-CORTE (2016), 12%. The NRC (2001) uses k_{preg} suggested by Ferrell et al. (1976). Metabolizable energy for pregnancy estimated in this study follows the same pattern of accretion as NEpreg, surpassing the NRC (2001) values at the end of the gestational period.

The estimated k_{preg} is lower than any other efficiencies of energy utilization (k_m and k_g). This inefficiency is probably because of the energetic cost associated with maintenance of pregnancy products (gravid uterus and mammary gland), which may be related to oxidative metabolism (Bauman and Currie, 1980). A great part of the energy available for pregnancy is expended as HP (Bauman and Currie, 1980), or with greater rates of muscular turnover, to offer amino acids as energy source to the fetus (Lage, 2015). Protein is the most abundant organic constituent of conceptus tissues (Ferrell et al., 1976). Therefore, according to Hammond (1947) the homeorhetic effect in cows, mainly pregnant, is a mechanism able to direct nutrients to tissues with high metabolic rates, as the gravid uterus, improving the energy parturition for fetal development.

Another important point to consider is the precise moment when pregnancy requirements should be added to dietary requirements. After estimating energy requirements for pregnancy, we determined the day of pregnancy when pregnancy requirements were statistically different from non-pregnant cows using the lower confidence limit of the retained energy in the gravid uterus ($P < 0.05$). Our data indicate that energy requirements for pregnancy should be accounted from d 70 of pregnancy onwards (Figure 8). It is well documented in the literature that fetus development begins before day 190 of pregnancy, initiating essential processes as organogenesis and myogenesis (Du et al., 2010; Funston et al., 2010), thus nutritionist should consider pregnancy requirments from d 70 of pregnancy onwards.

CONCLUSION

In conclusion, maintenance requirements for non-pregnant Holstein × Gyr cows are different from pregnant cows. The efficiency of use of ME for maintenance for Holstein × Gyr cows is lower than the recommendations of NRC (2001). Additionally, we recommend using data from non-pregnant animals to estimate energy requirements for gain. Furthermore, we believe that the proposed non-linear equation to estimate net energy requirements for pregnancy are more adequate than current NRC equation, and should be recommended for Holstein × Gyr. Lastly, our data suggests the beginning of pregnancy requirements from day 70 of pregnancy, thus we assumed this point as the beginning of biological need for nutrients from the fetus.

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Table 1. Metabolizable and net energy requirements for pregnant Holstein × Gyr cows.

Days of pregnancy	**Net energy for pregnancy (kcal/d)**		**Metabolizable energy for pregnancy (kcal/d)**	
	This study[1]	Ferrell et al., 1976[2]	This study	Ferrell et al., 1976
100	87	36	583	257
130	133	74	890	527
160	204	143	1358	1021
190	311	263	2074	1879
220	475	457	3166	3264
250	725	752	4833	5371
280	1107	1167	7377	8336

[1] Energy requirements for pregnancy calculated according to the estimated equation in this study. Efficiency of utilization of metabolizable energy for pregnancy = 15%. Net energy for pregnancy denotes energy retained in gravid uterus and mammary gland.

[2] Energy requirements for pregnancy adapted from Ferrell et al. (1976). Efficiency of utilization of metabolizable energy for pregnancy = 14%. Net energy for pregnancy denotes energy retained in gravid uterus and mammary gland.

FIGURES

Figure 5. Experimental scheme, feeding regimes and slaughter groups.

Figure 2. Components of gravid uterus and mammary gland (A) and maternal tissues' sub-divisions according to sampling at slaughter (B).

Figure 3. Representation of heat production (HP) equation for non-pregnant and pregnant cows. Close symbols refer to non-pregnant cows and open symbols refer to pregnant cows.

Figure 4. A - Estimation of net energy for maintenance requirement from NRC (2001) equation and the estimated equation from non-pregnant and pregnant Holstein × Gyr cows (this study). B – Estimation of metabolizable energy for maintenance requirement from NRC (2001) equation and the estimated equation from Holstein × Gyr cows (this study). Closed and open circles refer to values obtained from this study and closed triangles refer to values obtained from NRC (2001).

Figure 5. Relation between retained energy in empty body weight (EBW, Mcal/d) for non-pregnant cows or in maternal tissue (CT, Mcal/d) for pregnant cows and empty body gain (EBG, kg/d) for non-pregnant or maternal tissue gain (CTG, kg/d) for pregnant cows. Close circles refer to pregnant cows and open circles refer to non-pregnant cows.

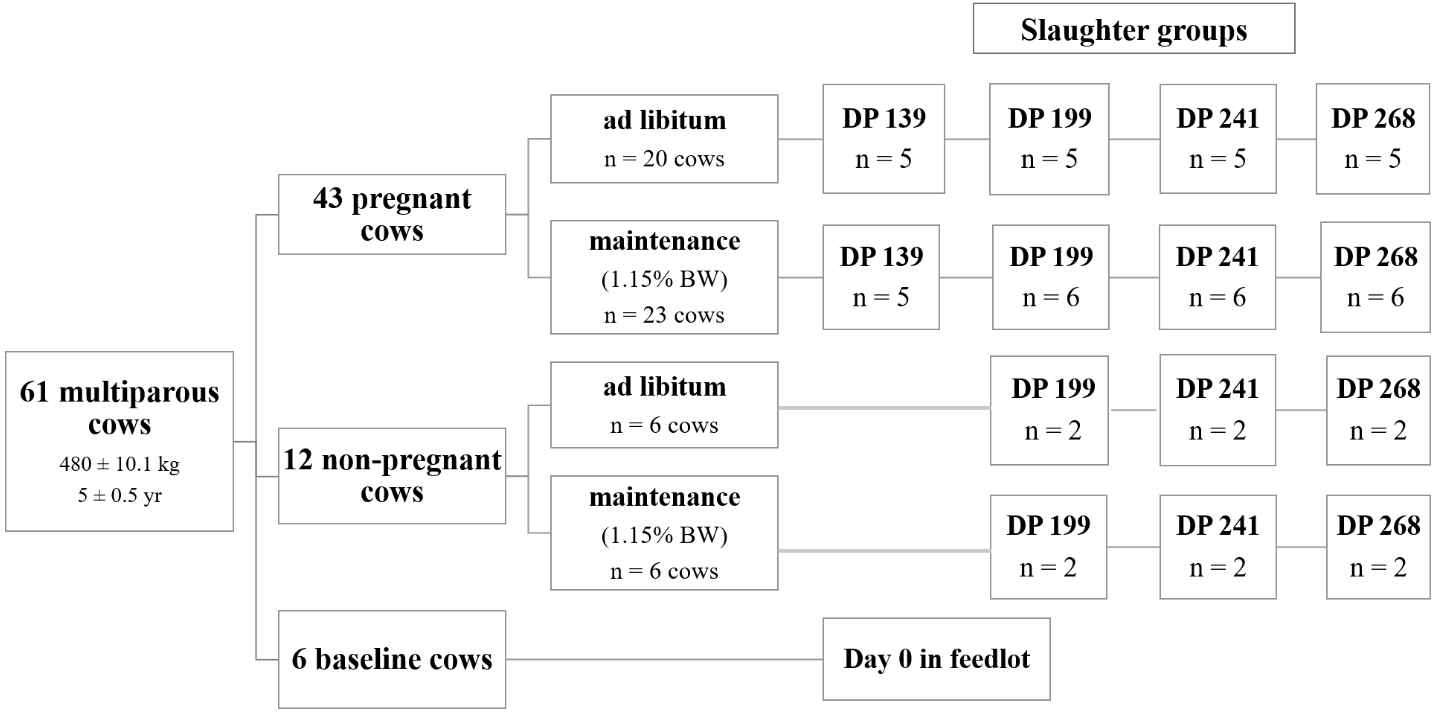
Figure 6. A - Estimation of net energy required for pregnancy (i.e., energy retained) by the NRC (2001) equation and the non-linear equation estimated in this study. The NRC (2001) equation for net energy retained (Mcal/d) is: (0.00318 × DP – 0.0352) × (CBW/45), where DP is days of pregnancy and CBW (kg) is calf birth weight. B – Relation between retained energy in the gravid uterus plus mammary gland and days

of pregnancy. Open symbols refer to cows fed ad libitum and closed symbols refer to cows fed at maintenance level.

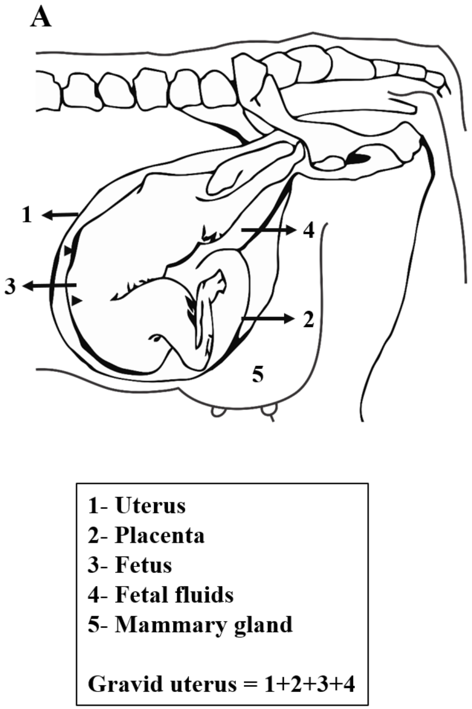
Figure 6. C - Estimation of metabolizable energy required for pregnancy (i.e., energy retained) by the NRC (2001) equation and the non-linear equation estimated in this study. The NRC (2001) equation for net energy retained (Mcal/d) is: $[(0.00318 \times DP - 0.0352) \times (CBW/45)]/0.14$, where DP is days of pregnancy and CBW (kg) is calf birth weight.

Figure 7. Pattern of retained energy in the gravid uterus and mammary gland according to days of pregnancy.

Figure 8. Initial point of energy requirement for pregnancy.



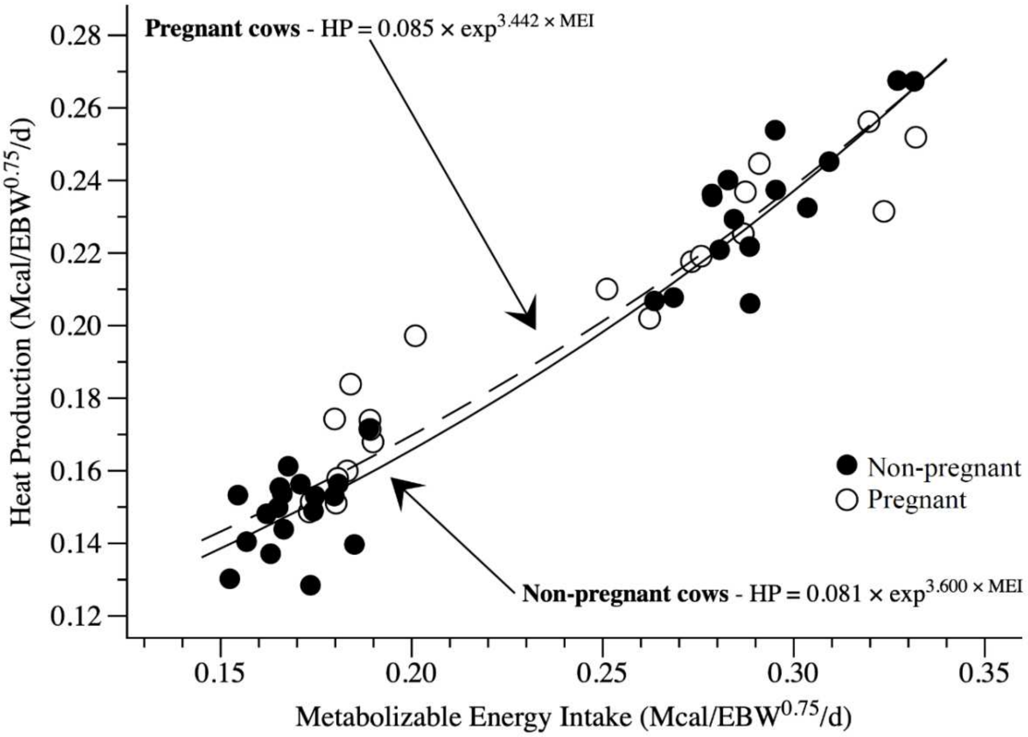
Sguizzato, figure 6.



B

Cow	**Carcass composition**	Bones and meat cuts from half carcass
	Non-carcass composition	Organs, stomachs, intestines, head, tail, hooves, trimmings, hide and blood.
	Maternal tissue	Sum of carcass and non-carcass components
	Mammary gland	The mammary gland with its skin, parenchimal, adipose tissue and colostrum.
	Gravid uteurus	The sum of uterus, placenta, fetus and fetal fluids.

Sguizzato, figure 2 A and B.



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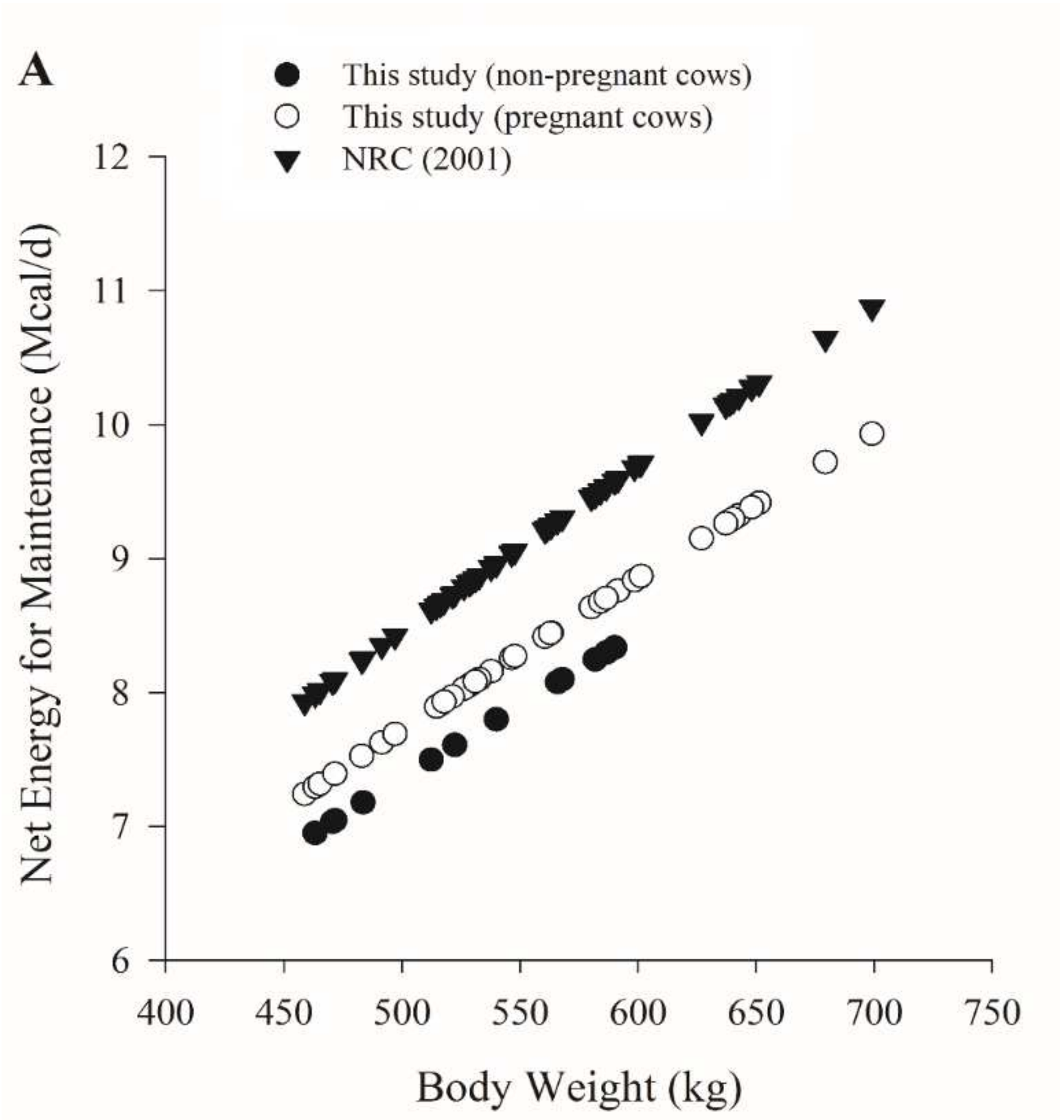

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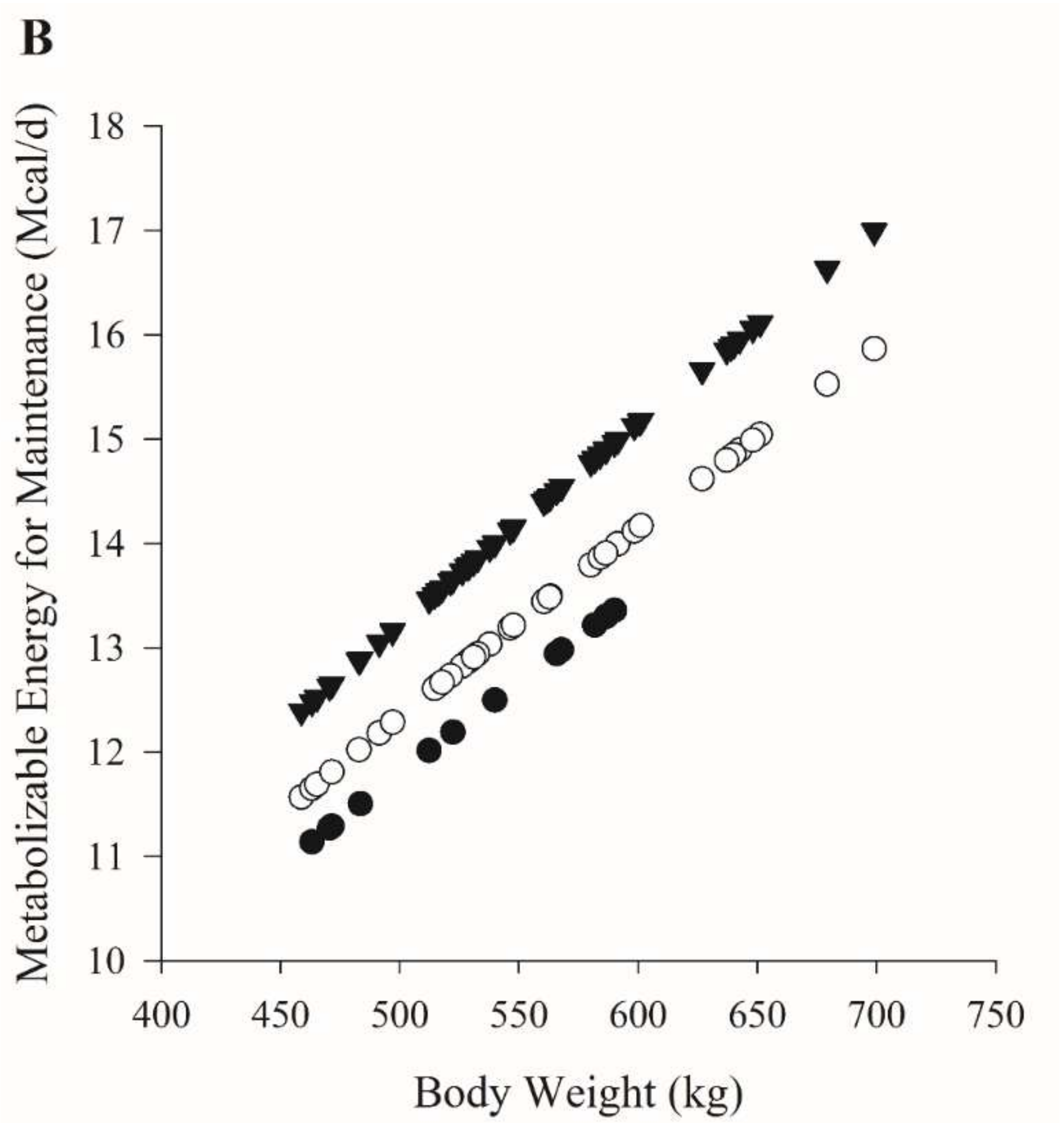

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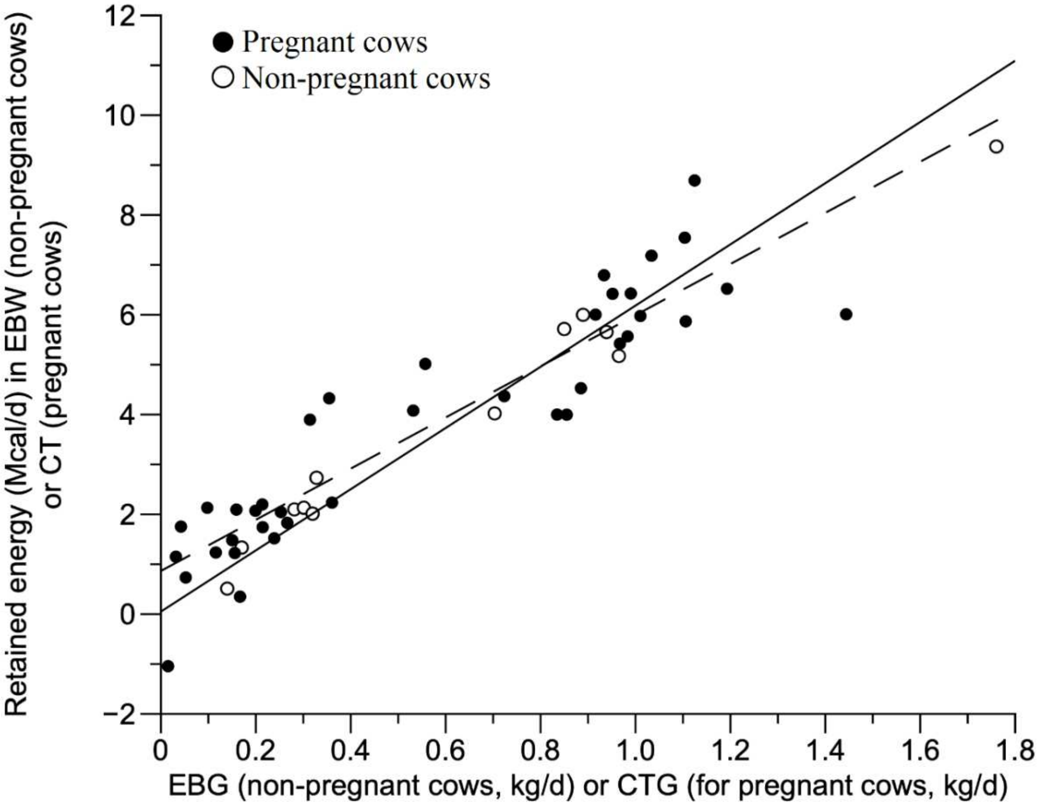

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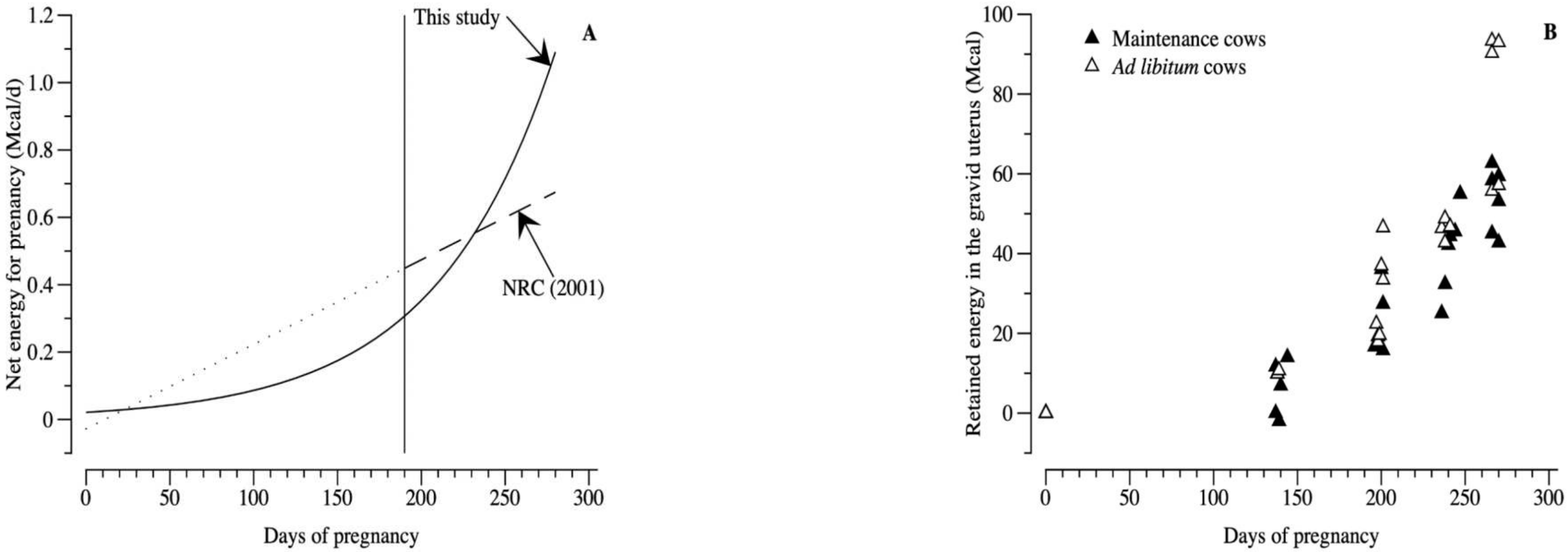
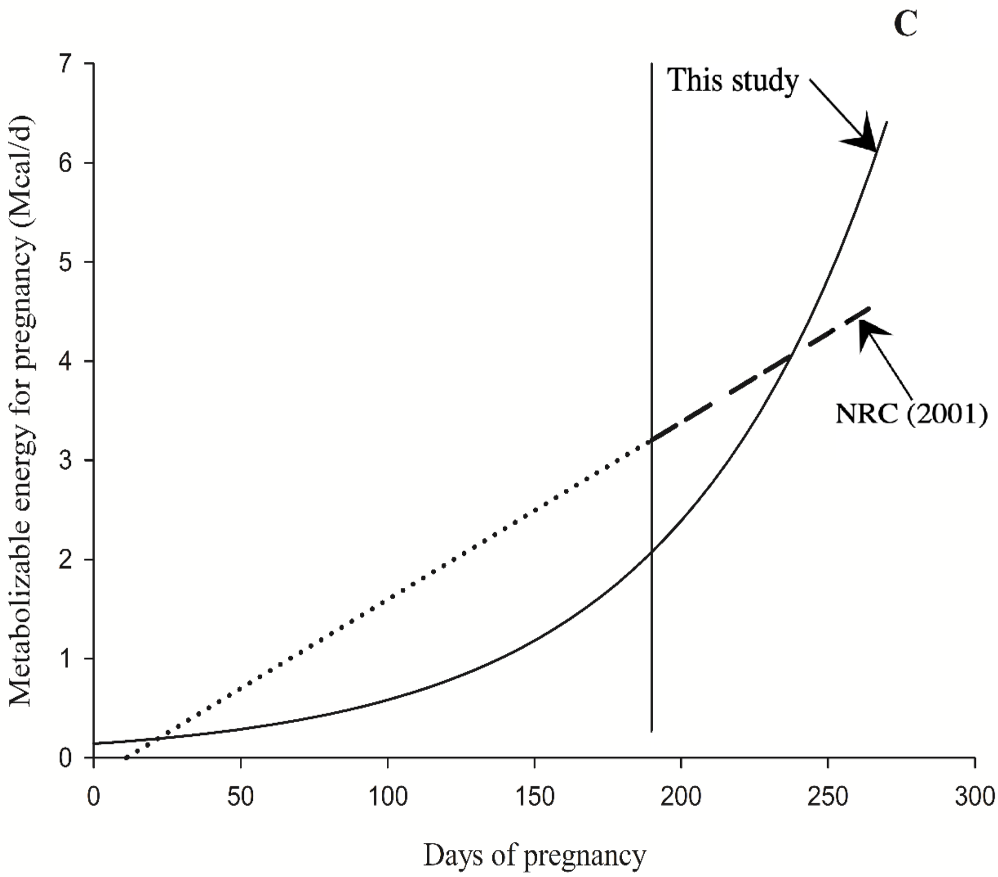


Sguizzato, figure 6 A and B.



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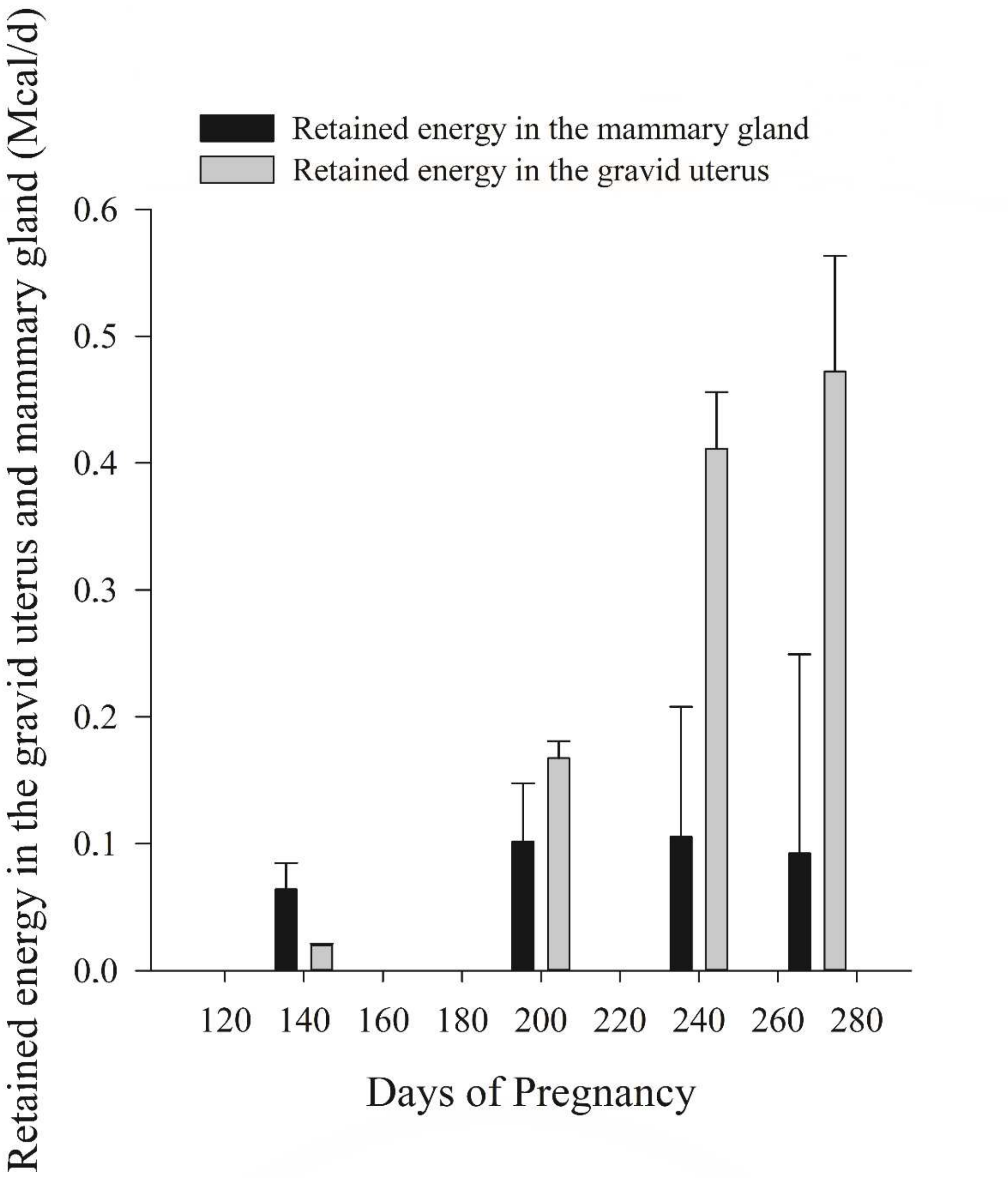

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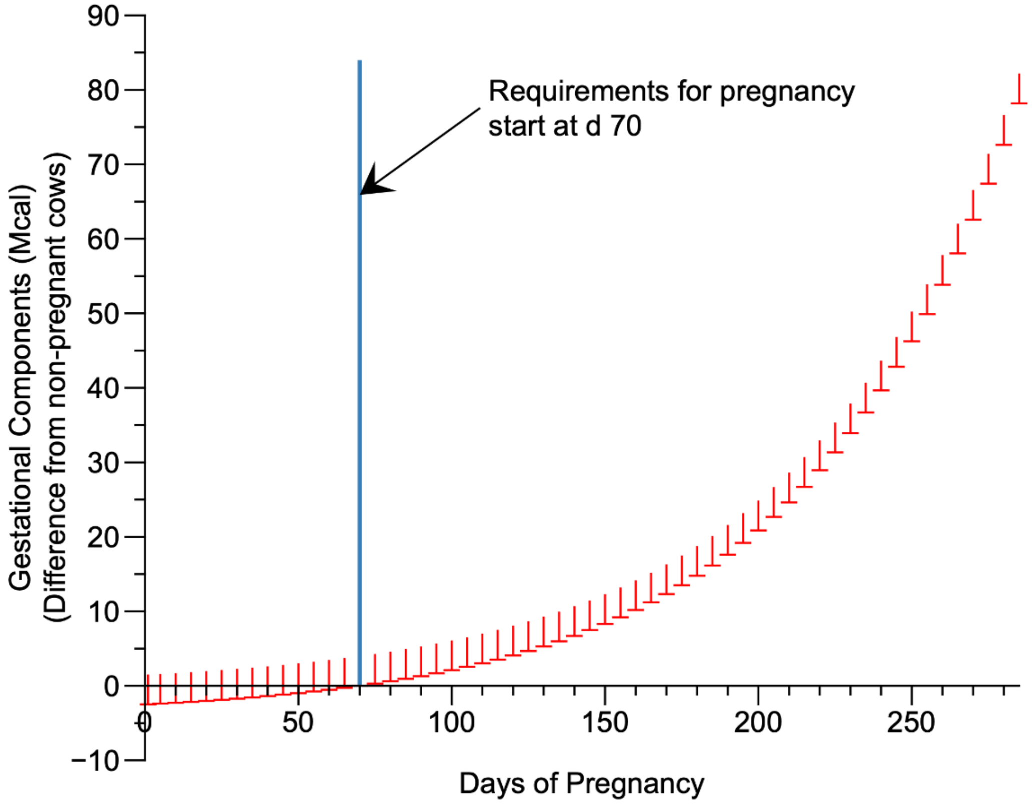

Sguizzato, figure 8.